syngenta

JUL 22 2004

Charles Breckenridge, Ph.D.
Sci. and Tech. Sr. Res. Fellow
P.O. Box 18300
Greensboro NC 27419-8300

Telephone: (336) 632-7082
Email: charles.breckenridge@syngenta.com

July 19, 2004

Dr. C. W. Jameson
National Toxicology Program Report on Carcinogens
79 Alexander Drive
Building 4401, Room 3118
PO Box 12233
Research Triangle Park, NC 27709
jameson@niehs.nih.gov

Subject: Department of Health and Human Services. Public Health Service. National Toxicology Program; Call for Public Comments on 21 Substances, Mixtures and Exposure Circumstances Proposed for Listing in the Report on Carcinogens, Twelfth Edition. Federal Register Notice Vol. 69, No. 97/Wednesday, May 19, 2004; 28940 – 28944.

Dear Dr. Jameson:

The subject Federal Register (FR) Notice calls for public comments on atrazine, one of the 21 substances proposed for listing in the 12th Report on Carcinogens (RoC). As a manufacturer of atrazine which is regulated by the US Environmental Protection Agency (EPA, the Agency), Syngenta Crop Protection, Inc. is submitting comments on the subject FR Notice. Atrazine does not meet the criteria for being a known or reasonably anticipated to be a human carcinogen and should be withdrawn from the list being considered for the 12th RoC. Syngenta also requested a review of the rationale for the proposed listing by Dr. H.B. Matthews, consultant in Toxicology and formerly a member of the National Toxicology Program's (NTP) Review Group One (Attachment 1).

The FR Notice contains two criteria for listing in the RoC which are: I. Chemicals that are known human carcinogens or may be reasonably anticipated to be human carcinogens, and II. Chemicals to which a significant number of persons residing in the United States (US) are exposed (including the nature of exposures, the estimated number of persons exposed and the extent to which the implementation of Federal regulations decreases the risk to public health from exposure).

Atrazine does not meet the RoC criteria for cancer listing category "known to be human carcinogens" (Criteria 1A) which states "There is sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent, substance or mixture, and human cancer."

Comprehensive reviews of the epidemiological literature, performed by EPA, IARC and independent experts indicate, that there is no basis for concluding that there is a causal association between exposure to atrazine and cancer in humans. Cohort studies conducted at the atrazine production facility (St. Gabriel) over a long period of follow up have not identified any increased cancer risk, including non-Hodgkin's lymphoma or prostate cancer. In addition, a study of a large cohort of licensed pesticide applicators in the Agricultural Health Study found no evidence of increased prostrate cancer incidence.

syngenta

A review of the case-control studies conducted by the National Cancer Institute, principally on non-Hodgkin's lymphomas (NHL), has not established a causal association between atrazine use and the occurrence of NHL. This conclusion has also been reached in two authoritative reviews ((http://www.epa.gov/oppsrrd1/reregistration/atrazine/ and IARC-Attachment 2) and by three independently published reviews (Delzell et. al, Attachment 3).

The criteria for listing as "may be reasonably anticipated to be human carcinogens" (Criteria 1b) are defined by the NTP as:

1) There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or,

2) There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or,

3) There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans."

Syngenta concludes that:

1) The aforementioned reviews of the epidemiological literature indicate that there is no credible evidence of a causal association between human cancer and atrazine exposure (Delzell, et al., 2004-Attachment 3). This conclusion was reached independently by EPA and IARC.

2) In evaluating the effects of atrazine on mammary tumor development in the female SD rat, an experimental basis has been established for the cascade of endocrine-related changes beginning with luteinizing hormone (LH) surge suppression, followed by estrous cycle disruption and leading to an earlier appearance and/or a higher incidence of fibroadenomas and adenocarcinomas. This pattern of endocrinologic aging has been extensively described for the female SD rat. High doses of atrazine accelerate the normal reproductive aging process in this strain of rat. Furthermore, the response observed in the female SD rat is unique to this strain, since neither the Fisher-344 rat nor 3 strains of mice have demonstrated any tumorigenic effect in lifetime bioassays (Attachment 4). This mode of action was judged by both EPA and IARC not to be operative in man.

syngenta

3) Neither atrazine nor its chlorotriazine metabolites are structurally related to any agent known to be a human carcinogen or reasonably anticipated to be a human carcinogen by NTP.

With regard to either classification noted above, the NTP website further states "Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans." [http://ntp-server.niehs.nih.gov/NewHomeRoc/ListingCriteria.html]

These supplemental criteria have been address in the 4th Expert Panel Report (Attachment 4), the updated weight of evidence assessment (Attachment 5) and by EPA's Interim Re-Registration Eligibility Decision ((http://www.epa.gov/oppsrrd1/reregistration/atrazine/).

These assessments indicate that

- Atrazine is not genotoxic, directly estrogenic or androgenic
- Atrazine does not induce aromatase in the intact animal
- Key events underlying the occurrence of mammary tumors in the female Sprague-Dawley rat are well defined.
- The sequence of events leading to mammary tumor development in female SD rats is temporally coherent.
- The mode of action is biologically plausible for the rat but not relevant for humans
- A dose-response assessment has been conducted for each key event in the female SD rat;
- The biological basis for the existence of a threshold for the critical key event (estrous cycle disruption) observed in the SD rat has been defined.
- The mode of action which cause tumors in SD rats has been defined and is not considered to be relevant for humans.

syngenta

- Human exposure at environmental concentrations are several orders of magnitude below the no observed effect level in the female SD rat (See Figure 1, page 15).

This assessment is consistent with position taken by EPA in the Interim Reregistration Eligibility Decision for atrazine as summarized below.

On October 31, 2003 the EPA completed and Interim Reregistration Eligibility Decision (IRED) (http://www.epa.gov/oppsrrd1/reregistration/atrazine/) for atrazine that contained evaluations of approximately 10 years of extensive scientific research on atrazine's safety, including numerous studies on the carcinogenic potential for atrazine and covering two separate Scientific Advisory Panel (SAP) meetings on the subject.

All studies submitted by Syngenta into the EPA Atrazine/Simazine Special Review docket [EPA Docket OPP30000-60] should be considered part of the administrative record for this proceeding and will be made available to NTP reviewers upon request (Attachment 6).

EPA Conclusions

EPA concluded that atrazine is not likely to cause cancer in humans. The weight of the animal evidence summarized at the 2000 SAP meeting on atrazine classification and updated here (Breckenridge and Stevens, 2004-Attachment 5) convincingly demonstrated that atrazine induced mammary tumors were unique to the Sprague-Dawley rat, and that the mode of action was not relevant to man.

In October, 2003, EPA completed a review and summary of scientific studies related to atrazine and cancer epidemiology including a consideration of animal mode of action related to selected cancers. The review includes an examination of animal mode of action issues related to prostate and ovarian cancers and non-Hodgkin's lymphoma. As discussed in the January 31, 2003 EPA Interim Reregistration Eligibility Decision, atrazine's mode of action (i.e., a decrease LH surge, failed ovulation and estrous cycle disruption) for induction of mammary gland tumors (the only tumor observed in animal bioassays) in SD female rats is not considered relevant to humans (http://www.epa.gov/oppsrrd1/reregistration/atrazine/).

In addition, there is no compelling or sufficient epidemiological evidence to conclude that a causal relationship between exposure to atrazine and human cancer exists (Delzell et al., 2004). This conclusion is supported by two independent authoritative reviews (http://www.epa.gov/oppsrrd1/reregistration/atrazine/; IARC, 1999; Attachment 2).

syngenta

Discussion of EPA Findings

Some of the details of the EPA review follow. The EPA's FIFRA Scientific Advisory Panel (SAP), convened in June 2000 determined that it is unlikely that atrazine's cancer mode of action in the SD rat is operative in humans (http://www.epa.gov/scipoly/sap/2000/#062700). HED's Cancer Assessment Review Committee (CARC) also concluded (December 13, 2000) that the mode of action is not relevant to humans (Attachment 7). On April 16, 2002 EPA's Health Effects Division (HED) issued its Revised Human Health Risk Assessment for the Reregistration Eligibility Decision for atrazine. EPA concluded that "In accordance with the *1999 Draft Guidelines for Carcinogen Risk Assessment*, the CARC classified atrazine as 'not likely to be carcinogenic to humans.'" The attenuation of the LH surge and estrus cycle disruptions appears to be a species, strain and sex specific effect occurring only in female Sprague-Dawley rats (http://www.epa.gov/oppsrrd1/reregistration/atrazine/).

"While atrazine is associated with mammary and pituitary tumors in female Sprague-Dawley (SD) rats, this is not the case in male SD rats, or either sex of Fischer 344 (F-344) rats or CD-1 mice. Mutagenic and estrogenic activity do not appear to play a significant role in atrazine-associated carcinogenicity. Biological plausibility has been established for the mode of carcinogenic activity of atrazine. The rat cancer mode of action (MOA) involves a process consisting of modulation of the gonadotrophin releasing hormone (GnRH) pulse, attenuation of pituitary releases of luteinizing hormone (LH), and alteration of ovulatory cycles, expressed as constant estrus, which leads to prolonged exposure of mammary and pituitary tissues to estrogen and prolactin, and development of tumors in response to then prolonged hormone exposures. This MOA essentially accelerates the normal aging process in female SD rats. It would be expected to be operative in other rat strains with a similar reproductive aging process (e.g. Long Evans and Wistar). Although atrazine might cause adverse effects on hypothalamicpituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen and prolactin) that is found in SD rats is not expected to occur in humans. Instead, humans respond to reduced LH by having reductions in estrogen and prolactin" (Attachment 7).

The available epidemiological evidence on atrazine does not provide credible evidence of a causal link between atrazine exposure and human cancer (Delzell et al., 2004, http://www.epa.gov/oppsrrd1/reregistration/atrazine/; IARC, 1999; Attachment 2). Neither of the two most extensively investigated cancers, prostate and non-Hodgkin's lymphoma, have provided adequate evidence to draw a credible interpretation of causation. For prostrate cancer, the nested case-control study reported by Hessel et al., (2004) provided convincing evidence that the excess prostate cancer incidence in workers at the St. Gabriel production facility was fully accounted for by the PSA screening bias. The Agricultural Health (Alavanja, 2003) study did not find any evidence of an association between reported atrazine use and prostrate cancer in a large cohort of licensed applicators from North Carolina and Iowa; all associations, include trend analysis, favored the null hypothesis.

For non-Hodgkin's lymphoma, the cancer incidence study conducted at St. Gabriel has not revealed an excess of non-Hodgkin's lymphomas among employees working in jobs that would bring them in contact with atrazine (MacLemann, 2002). The series of studies conducted by the National Cancer Institute in the late 1980s and early 1990s have not provided convincing evidence of a causal association between atrazine use and the occurrence of non-Hodgkin's lymphoma. In spite of two attempts at more refined analysis (Hoar, 1993, de Roos, 2003), the basic results remain unconvincing, as concluded by IARC, the EPA Science Advisory Panel and EPA that reached the decision that atrazine was not likely to be a human carcinogen.

Additionally in 2003 EPA (Attachment 8) concluded that "Studies of manufacturing and farming populations do not support a finding that atrazine is a likely cause of prostate cancer" (http://www.epa.gov/oppsrrd1/reregistration/atrazine/); Delzell, et al. 2004; Attachment 3).

After a thorough review of all available studies the following EPA conclusion was made: "The Agency does not find any results among the available studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine" (http://www.epa.gov/oppsrrd1/reregistration/atrazine/).

"Even though the epidemiological evidence and animal data, when viewed separately, do not support a positive cancer finding for atrazine, EPA examined the totality of animal and human data to determine if that approach showed that atrazine was likely to cause a carcinogenic response in humans. Specifically, EPA reviewed the available animal data to determine if a mechanism could be identified which supports the biological plausibility of atrazine as a human carcinogen taking into account the tumors that were identified in the epidemiological data. This review showed that (1) lymphomas, including NHL, were generally not seen in atrazine animal bioassays; (2) a mechanistic role for atrazine contributing to NHL has not been identified in laboratory studies; (3) tumors at any endocrine site other than mammary gland tumors in female SD rats (e.g., prostate, ovarian tumors) have not been identified in atrazine bioassays; (4) the SAP concluded in 2000 that the mammary gland tumors in rats caused by atrazine are produced via a mechanism not relevant to humans; and (5) the endocrine tumors that have been raised in epidemiological studies (other than mammary gland tumors) can not be biologically tied to atrazine's mode of action (i.e., decrease prolactin, decrease luteinizing hormone (LH) and suppression of ovulation). Thus, at this time, joint consideration of the available animal cancer and mode of action data and epidemiological studies, does not indicate that atrazine is likely to cause cancer in humans" (http://www.epa.gov/oppsrrd1/reregistration/atrazine/).

Other Reviews

syngenta

In addition to the EPA reviews, the International Agency for Research on Carcinogens (IARC), the National Registration Authority for Agricultural and Veterinary Chemicals of Australia, and the United Kingdom Pesticide Directorate also reviewed atrazine and found it to be safe to humans and the environment. Their reviews are summarized below:

The International Agency for Research on Carcinogenicity Review

The information included in the FR Notice and on the NTP website [http://ntp-server.niehs.nih.gov/NewHomeRoc/AtrazineSumm.html] indicates that the National Institute of Environmental Health Sciences' (NIEHS) basis for nomination of atrazine is "IARC[2] finding of sufficient evidence of carcinogenicity in animals (Vol. 73, 1999) [[2]International Agency for Research on Cancer (IARC)]." The IARC concluded that while there was sufficient evidence for carcinogenicity in the SD rat, after considering the atrazine mode of action research, the IARC concluded that there was strong evidence that the mechanism responsible for mammary tumor formation the Sprague-Dawley rat is not relevant to humans. The IARC review concludes: "Therefore, there is strong evidence that the mechanism by which atrazine increases the incidence of mammary tumors in Sprague-Dawley rats is not relevant to humans and categorizes atrazine as "not classifiable as to carcinogenicity to humans (Group 3)" (Attachment 2).

Australia

A regulatory review in Australia determined that data regarding the formation of tumors in the Sprague-Dawley rat exposed to high levels of atrazine has no relevance to humans because the pattern of estrogen levels in aging Sprague-Dawley rats differs from that of other rats and from that in humans. The NRA Review of Atrazine: Existing Chemical Review Program, National Registration Authority for Agriculture and Veterinary Chemicals of Australia; November 1997, 3 Volumes. Submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597607).

EU

A review conducted for the European Union by regulatory officials in the United Kingdom concluded that, "It is expected that the use of atrazine, consistent with good plant protection practice, will not have any harmful effects on human or animal health or any unacceptable effects on the environment." Atrazine: Report and Proposed Decision of the United Kingdom Made to the European Commission Under Article 7(1) of Regulation 3600/92 Council Directive 91/414/EEC Regulation 3600/92 3 Volumes. Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315415).

syngenta

Exposure

The scientific data on atrazine clearly does not support the carcinogenicity criteria for listing atrazine in the RoC, therefore exposure information is secondary to the listing question. In addition human exposure at environmental concentrations are several orders of magnitude below the no observed effect level in the female Sprague-Dawley rat.

Nature of Exposure

Dietary Exposure

Chronic dietary exposure assessments were performed for atrazine and its chlorinated metabolites by EPA's HED. As per OPP policy, a reference dose (RfD) modified by an FQPA safety factor is referred to as a population adjusted dose (PAD). An additional FQPA safety factor was retained as 10X to be applied to dietary risk assessments for atrazine. Therefore, the chronic RfD used in the assessment has a thousand-fold safety factor applied to the No Effect Level of the most sensitive endpoint was 0.018 mg/kg/day, and the chronic population adjusted dose (cPAD) was 0.0018 mg/kg/day. All chlorotriazine metabolites were included in the risk assessment. Average exposures less than 100% of the cPAD are below HED's levels of concern for chronic effects. Risk estimates for all subgroups analyzed were less than 1% of the chronic population adjusted dose (cPAD), and therefore risk estimates for all subgroups are below HED's level of concern.

Hydroxyatrazine was not mutagenic or carcinogenic in the Sprague-Dawley rat. A unique toxicological endpoint (kidney damage due to precipitation of hydroxyatrazine in the urine) was used to establish the chronic reference dose for hydroxyatrazine. A chronic dietary exposure assessment was performed for the hydroxy-metabolites of atrazine. Available data on exposure to hydroxyatrazine is limited to the oral route. Because the FQPA safety factor was reduced to 1X for the hydroxy-metabolites of atrazine, the chronic RfD for the hydroxyatrazine (0.01 mg/kg/day) is equal to the chronic PAD. All population subgroups had exposures below their respective cPADs. Risk estimates for all subgroups analyzed were less than 1.0% of the chronic population adjusted dose (cPAD), and therefore risk estimates for all subgroups are below HED's level of concern.
[http://www.epa.gov/oppsrrd1/reregistration/atrazine/hed_redchap_16apr02.PDF;]

syngenta

Exposure through Drinking Water

Syngenta has extensive atrazine exposure data that has been compiled and provided to the EPA over the years. The major route of exposure to atrazine and its chlorometabolites is via water. However the extent to which concern for this route of exposure occurs must be determined based on whether the levels are considered to be harmful if ingested, not simply on their presence. The US EPA has developed a series of standards that provide guidance for what is an acceptable exposure from a health effects perspective. Below, these standards are discussed and the extensive drinking water monitoring database for atrazine is also summarized.

Drinking Water Standards

There are two sets of drinking water guidance in the US for atrazine. Under the Safe Drinking Water Act (SDWA), the EPA-Office of Water (OW) developed short term (one and ten day), long-term (about 7 years or ~10% of an individual's lifetime) and lifetime (~70 years of exposure) Health Advisory Levels (HAL) for atrazine. A regulatory Maximum Contaminant Level (MCL) was also promulgated under the SDWA in 1991 for only parent atrazine. The atrazine HAL and MCL are found in the EPA-OW publications entitled Drinking Water Standards and Health Advisories (2002 Edition EPA 822-R-02-038) as well as in previous editions. These existing EPA-OW drinking water HALS and MCL are listed in the following table. Based on EPA's recent scientific reviews of atrazine, the MCL is expected to be increased. The lifetime MCL was developed with a 1000-fold safety factor and allows for 20% of exposure to come from water.

Health Advisory Levels (HAL) and Maximum Contaminant Level (MCL) For Atrazine

Exposure Type	HAL (ppb)	MCL (ppb)	Safety Factor
1-Day, Child	100	-	100
10-Day Child	100	-	100
7-Year, Child	50	-	100
70-Year, Adult	3[a]	3	1000

[a]70 year HAL = MCL

A second set of drinking water criteria have recently been developed by EPA Office of Pesticide Programs (OPP) as part of the Interim Reregistration Eligibility Decision (IRED) for Atrazine released in January 31, 2003. This EPA-OPP health-based review provided a more refined drinking water levels of comparison that included parent atrazine and the three chlorinated metabolites: deethylatrazine,

syngenta

deisopropylatrazine, and diaminochlorotriazine. The summation of parent atrazine and three metabolites is termed Total Chloro-Triazine (TCT). EPA-OPP developed Drinking Water Levels of Comparison (DWLOC) for six subpopulations as noted in the following table.

Summary of Lowest Drinking Water Levels of Comparison (DWLOC) for Atrazine and its Chlorinated Metabolites from EPA-OPP Interim Registration Eligibility Decision (IRED), January 31, 2003

	Intermediate (Seasonal) Chronic (Annual) Exposure (ppb)	
Population Subgroup	**90-day**	**Annual**
General Population	-	68
Infants <1 year old	12.5/37.5	12.5
Children 1 to 6	-	23
Children 7 to 12	-	53
Females 13 to 50	-	60
Males 13 to 19	-	68
Males 20 and over	-	68
Seniors	-	68

A Drinking Water Level of Comparison (DWLOC) for an acute exposure period of one day was developed for the six subpopulations. The lowest acute DWLOC was 298 ppb for the female subpopulation (age 13-50). The other five subpopulations had acute (one day) DWLOC greater than 298 ppb.

EPA-OPP developed a DWLOC for TCT at 12.5 ppb for the subpopulation of infants less than one year evaluated over an intermediate exposure based on a 90-day rolling average. It further established, based on an enhanced annual monitoring frequency, a DWLOC of 37.5 ppb in raw water at the intake structure of the CWS and on surface water with a 90 day rolling average.

EPA-OPP also developed chronic DWLOC for five of the six subpopulations as noted in the table. The DWLOC ranged from 23 ppb TCT for subpopulation of children (age 1-6) to 68 ppb TCT for males (13 and over) evaluated on an annual exposure period. All chronic DWLOC as well as the DWLOC for infants less than one year of 12.5 ppb were developed with a 1000 fold safety factor. The LH endpoint used to develop the DWLOC is not relevant to infants. The most sensitive endpoint relevant to infants is a NOEL of 6.2 mg/kg/day which is 3.5 times higher than the LH NOEL of 1.8 mg/kg/day adding another 3.5X safety factor to the standards.

syngenta

Atrazine Health-Based Drinking Water Criteria Outside the United States

Australia estimated a chronic (lifetime) drinking water level of 20 ppb TCT (sum of parent atrazine and three chlorometabolites) as part of an atrazine review in 1997.

United Kingdom conducted a reregistration review for the European Union in 1996 and developed a chronic (lifetime) health-based drinking water level of 15 ppb for parent atrazine. The EU has proposed to discontinue use of all pesticides including atrazine found in groundwater at concentrations greater than 0.1 ppb even when concentrations were attributed to uses no longer registered and science reviews are favorable. The 0.1 ppb regulatory standard is neither health-based nor scientifically supported. A triazine herbicide similar to atrazine continues to be used in the EU for weed control in corn.

Summary of Atrazine Exposure in Ground and Surface Water

Comprehensive monitoring data are available for atrazine in ground and surface water in the US (Attachment 9).

Summary of Atrazine in Ground Water

- In GW CWS, chronic long term atrazine levels have never exceeded EPA's Atrazine MCL of 3 ppb and 99 percentile concentrations have been demonstrated to be significantly below EPA's MCL of 3 ppb for atrazine and DWLOC of 12.5 ppb for TCT.
- Exceedance of the atrazine MCL of 3 ppb and TCT DWLOC of 12.5 ppb in Syngenta's Rural Well program declined from 0.7 % for atrazine and 0.5% for TCT in 92-94 to 0% in 2001, indicating that exceedance of chronic atrazine and TCT levels would be extremely unlikely even in the areas of greatest atrazine use and highest well sensitivity.
- The frequency of exceedance in Syngenta's Rural Well sampling program was greater than in any other GW monitoring program conducted due to its deliberate selection of highly vulnerable settings– it is therefore a conservative worst case for estimating potential exposure through rural wells.
- Exceedance of 3 ppb atrazine in less focused atrazine monitoring programs before the 1990 and 1992 label changes were effective was 0.1% or less, DWLOC exceedance is estimated at less than 0.1% - significantly lower than in Syngenta's worst case study – and should have further declined following the 1990/1992 label changes.
- Post 1995 exceedance of 3 ppb of atrazine in individual wells is 0.2% in ARP's worst-case edge-of-the field indicator – not drinking water - wells; exceedance of chronic TCT levels is extremely unlikely, since high percentile TCT levels are typically 2 times the corresponding atrazine concentration and none of the well years exceeded 6 ppb of atrazine.

syngenta

Atrazine Detection Percentiles in Community Water Systems on Groundwater. Below are summary statistics representing 14863 CWS on GW (418 CWS with one or more atrazine detects in 93-98, 14445 with no atrazine detects in 93-98) in atrazine use areas assessed in 2000.

Percentile	Atrazine [ppb] Percentile of CWS	Atrazine [ppb] Percentile of Population of Persons served	TCT [ppb] Percentile of CWS	TCT [ppb] Percentile of Population of Persons served
95th	0.0235	0.0627	0.1134	0.2713
97.5th	0.0673	0.1726	0.3420	0.4940
99th	0.1719	0.3668	0.5624	0.6244

Atrazine annual average and multi years period means in CWS on GW

	Year											Period
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Mean
Number of CWS w Data	3687	5851	7966	6538	7491	7225	5825	7496	5023	4858	7114	24505
Mean Annual Conc./ all CWS [ppb]	0.15	0.18	0.14	0.20	0.16	0.25	0.15	0.15	0.17	0.14	0.12	0.18
Max. Conc. P.a. [ppb]	4.20	5.26	5.56	10.40	2.88	3.63	2.60	3.80	1.53	1.87	2.10	2.72
Number of CWS Exceeding 3 ppb	1	7	4	8	0	2	0	2	0	0	0	0

Summary of Atrazine in Surface Water

- In the most vulnerable CWS on SW, concentrations declined over time and resulted in a 50% decrease of annual mean concentrations (3.7 to 1.8 ppb) indicating a reduction of environmental exposure during the 1994-2000 time period.
- In CWS on SW, mean concentrations in finished water decreased by more than 50% in the 1993-2003 period
- These data are consistent with USGS data that show atrazine levels in 53 Midwestern streams in 9 major use states decreasing about 50% between 89/90 and 94/95 – levels have continued to decrease in 98.
- There are **NO** active CWS with multi-year period means exceeding the lifetime MCL. No system has exceeded EPA's DWLOC since the 2003 monitoring program started.

syngenta

Atrazine annual average and multi year period means in CWS on SW

	Year										
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Number of SW CWS with SDWA Data	387	629	628	835	674	799	1001	856	1579	1392	1914
Mean Annual Conc./ all CWS	0.37	0.65	0.48	0.61	0.56	0.47	0.38	0.34	0.25	0.27	0.20
Number of CWS Exceeding 3 ppb	5	28	3	23	15	3	2	1	2	1	3

Notices of Violation for Atrazine MCL (3 ppb) Drinking Water Quarterly Standard under the SDWA (compiled from state government records)Drinking Water Standards

Out of the 54,000 CWS on ground or surface water in the US, 3 and 2 systems received notices of MCL violations for atrazine between 2000 and 2002. This low number of violations is attributed to atrazine watershed and label mitigation measures that were put in place in the early 1990s and were more fully adopted by growers after 1995 as well as changes in use patterns and stewardship practices.

Over an eight year period (1993-2000), the SDWA data shows three CWS on surface water out of 5,394 CWS with data in the 32 major use states had a period mean above 3 ppb ranging from 3.30 ppb to 3.41 ppb. Safety margins are greater than 1000 even in these watersheds vulnerable to atrazine. Currently there are no active CWS with multi-year period means greater than 3 ppb.

Syngenta Atrazine Monitoring Program (AMP) Under the EPA-OPP IRED and Memorandum of Agreement (MOA)

Syngenta has implemented an enhanced monitoring program for selected CWS on surface water as a condition of the EPA IRED and MOA. It was started in 2003. The monitoring program uses a conservative trigger to annually screen all CWS on surface water in the 50 states with atrazine monitoring data for potential inclusion in the atrazine monitoring program (AMP). The more frequent monitoring allows for an estimate of the 90-day rolling average triggers for atrazine and metabolites recently established by EPA OPP. This program supplemented the SDWA monitoring requirements for atrazine as carried out by CWS and administered by each state's lead drinking water agency. The screening trigger uses a conversion factor to calculate a TCT annual mean for each CWS from atrazine monitoring data collected under the SDWA each year. If a CWS

exceeds the trigger of a TCT annual mean of 2.6 ppb in, for example, in 2003; it would be included in the next year's (2004) program. This is a conservative trigger since an annual atrazine mean of 1.6 ppb (~½ of the MCL of 3 ppb) would convert to an annual TCT mean equal to or greater than 2.6 ppb for inclusion in the AMP. The SDWA data from 2002 was compiled from CWS with atrazine monitoring data representing 46 states. All CWS with monitoring data were included in the assessment. Eight CWS out of ~1,000 CWS on surface water in the 50 states had annual mean TCT concentrations at or above 2.6 ppb TCT trigger for potential inclusion in the AMP in 2003. None of these 8 CWS had an atrazine annual mean concentrations that exceeded the MCL of 3 ppb.

To date, none of the CWS in the atrazine monitoring program in 2003 approach or exceed the DWLOC concentrations.

Occupational and Residential Exposure

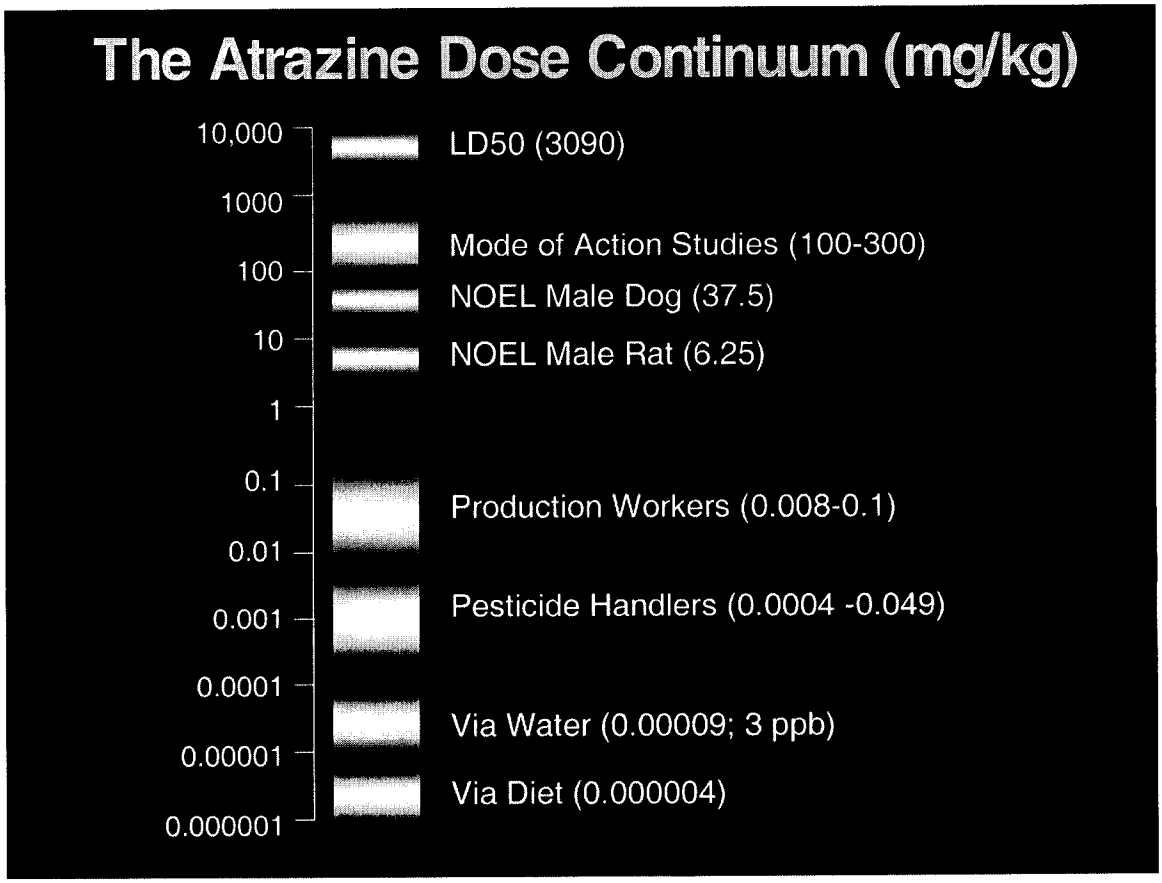
Based upon the results of EPA's occupational and residential exposure assessment for atrazine, and conditions stipulated by EPA in the IRED (January, 31, 2003 IRED), acceptable margins of exposure exist for all exposure scenarios.

syngenta

Magnitude of Exposure Relative to Toxicological No Observable Effect Levels (NOELS)

In Figure 1 provides the estimated dose continuum for exposure to atrazine in manufacturing and agricultural workers, in drinking water (overestimated at 3 ppb), and from all dietary sources. The data indicate that exposure to atrazine for the general public is 4 to 6 orders of magnitude lower than the NOELS from the most sensitive species in toxicity studies and 2 to 4 orders of magnitude lower than exposure for manufacturing and agricultural workers. At these levels of exposure, the EPA has determined in the Interim Re-registration Eligibility decision for atrazine that there would be no toxicological effects of atrazine.

Figure 1



syngenta

Extent to Which Federal Regulations Decreases the Risk to Public Health From Exposure

Extensive federal label changes have been implemented for atrazine since 1990 to reduce exposure. These include but are not limited to:

The groundwater related label changes made in 1990:

- Reduction in maximum rate allowed for non-cropland total vegetation control from 40 lbs. ai/a to 10 lbs. ai/a;
- Rate reductions in corn/sorghum (4 to 3 lbs ai/a);
- Restricted use classification for ground water concerns added (excluded uses for lawn care); 50 ft. well-head buffer added for mix/load/use.

In 1992 additional label changes were added with the aim to further reduce surface water detection levels. They included:

- Deleted non-cropland total vegetation control (@ 10 lbs. ai/a rate)
- Reduction in corn/sorghum rates from a maximum of 3 lbs. ai/a/year to 2.5 lbs ai/a/year (combined pre- and post-use) or maximum of 2.0 as single pre-emergence or post-emergence treatment;
- Setbacks from lakes, streams and reservoirs
- State/local preemption for more stringent requirements to allow use of localized best management practices (BMPs)

After a thorough science review atrazine meets the standards for reregistration in the U.S. Additionally, by implementing an intensive monitoring program when certain levels of atrazine are detected in water supplies, and by prohibiting atrazine uses in watersheds that result in exceedances, EPA will be able to ensure that exposures to atrazine in drinking water do not reach levels that pose a risk to public health. Study of rural wells will similarly continue to confirm that levels of exposure through groundwater are far below standards.

syngenta

Conclusions

Syngenta submits that atrazine does not meet the NTP criteria as an agent that is either "known to be human a carcinogen" or as an agent that "may be reasonably anticipated to be a human carcinogen because

- **Review of the epidemiological evidence by EPA, IARC and independent experts have not found any credible evidence of a causal association between atrazine exposure and human cancer.**
- **The mode of action underlying the carcinogenic response, observed only at high doses in a highly susceptible strain of rat, in one sex and one organ, is well understood with respect key events, dose response, and temporal consistency.**
- **The mode of action underlying the carcinogenic response observed in the female Sprague-Dawley rat was concluded by EPA and IARC to be not relevant to humans.**

Syngenta has fully characterized potential exposure to atrazine via diet, water and in the work place. Based on this data, EPA has granted the interim reregistration of atrazine and has concluded that acceptable margins of exposure exist for all registered uses. Figure 1 indicates that exposure to atrazine for the general public is 4 to 6 orders of magnitude lower than the NOELS from the most sensitive species in toxicity studies and 2 to 4 orders of magnitude lower than exposure for manufacturing and agricultural workers.

Based upon this assessment, Syngenta requests that atrazine be removed from further consideration for listing in the 12th ROC.

Sincerely,

Charles Breckenridge

Charles Breckenridge, Ph.D.
Sci. and Tech. Senior Research Fellow
Syngenta Crop Protection, Inc.

Attachment

List of Attachments

1. H. B. Matthews, Ph.D. July 19, 2004 Letter to Dr. C. W. Jameson Re: Nomination of Atrazine for Possible Listing in the Report on Carcinogens, Twelfth Edition.

2. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, October 13-20, 1998.

3. A Review of the Epidemiological Studies on Atrazine and Other Chlorotriazine Herbicides. Dr. Elizabeth Delzell, Dr. Jack Mandel and Dr. Charles Breckenridge.

4. Evaluation of a Hormonal Mechanism For Mammary Tumorigenesis of the Chloro-S-Triazine Herbicides: Fourth Consensus Panel Report: January 13, 2000. Prof. James Simpkins., Ph.D.

5. A Weight of the Evidence Evaluation of the Carcinogenic Potential of Atrazine Conducted According to USEPA's Draft Cancer Risk Assessment Guidelines. Dr. Charles Breckenridge and Dr. Jim Stevens.

6. Bibliography of Documents Submitted by Syngenta Crop Protection for the Atrazine and Simazine Special Review (Updated July 19, 2004).

7. Cancer Assessment Review Committee Atrazine Evaluation of Carcinogenic Potential. December 13, 2000. HED Doc. No. 014431.

8. Dr. Jerome Blondell October 28, 2003 Memo to Dr. Eric Olson Re: Review of Atrazine Cancer Epidemiology.

9. Exposure to Atrazine through Drinking Water. Dr. Peter Hertl.

July 19, 2004

Dr. C. W. Jameson
Report on Carcinogens
79 Alexander Drive
P.O. Box 12233
Research Triangle Park, NC 27709

Dr. Jameson:

At the request of Syngenta Crop Protection, Inc., Greensboro, NC I have completed a review of the nomination of atrazine for possible listing in the Report on Carcinogens, Twelfth Edition. My knowledge of facts pertaining to the carcinogenicity of atrazine was gained during my work with the USDA Office of Pest Management Policies (OPMP) and my recent review of the data. I worked with OPMP for much of the year that I served as the Society of Toxicology's Congressional Science Fellowship in Washington during the year 2000. An important part of my work with OPMP was to provide expertise on specific agricultural chemicals such as atrazine. My experience in review of chemicals for the Report on Carcinogens was gained in the course of the many years I worked for the National Toxicology Program during which I served as a member of Review Group One of the Report on Carcinogens.

As an expert on agricultural chemicals for the USDA, I carefully reviewed the "Preliminary Draft of the Hazard and Dose-Response Assessment and Characterization for Atrazine" prepared by the EPA as well considerable other information. I also attended the EPA Science Advisory Panel (SAP) on atrazine chaired by Dr. Chris Portier of NIEHS and the NTP. In my attendance of the Panel meeting and my review of the SAP report I was gratified to see that their conclusions were consistent with my own. Highlights of that SAP Report (No. 2000-5) that are highly relevant to a decision to consider atrazine for listing in the Report on Carcinogens are the following:

1. The members of the EPA SAP on atrazine were in agreement that high doses of atrazine caused an increase in mammary tumors in female Sprague Dawley (SD) rats in three of four bioassays, but not in male SD rats or in mice of either sex. Further, three chronic studies of atrazine using F344 rats were negative. Perhaps most important, the one chronic study that used ovariectomized SD rats failed to detect an increased incidence of tumors. (The one study that indicated atrazine treatment increased the incidence of mammary tumors in male F344 rats was shown to have used a flawed experimental design. The SAP concluded that "a close look at the study revealed that the animals in the high dose group in which tumors were reported to increase lived longer that the controls by several months. Since this strain shows a significant increase in the incidence of mammary tumors with age, the Panel concluded that the observed increase in tumors is due to the increased age of the high dose group and not due to atrazine.")

2. The mechanism thought to account for the increased incidence of tumors observed in SD rats was agreed to be selective endocrine modulation during their aging that does not occur in other strains of rats or in humans.
3. The mechanism involved in SD rats has been demonstrated through elegant research to involve the release of gonadotrophin releasing hormone (GnRH) from the hypothalamous. This release occurs during the afternoon pituitary luteinizing hormone (LH) surge to result in a lengthened estrus cycle. The resulting increased estrogen levels are thought to be the proximate cause leading to an increased incidence of tumors. This mechanism does not play a role in humans. It was strongly emphasized that the effects of age on reproductive and endocrine function are much different in humans than in SD rats. The mechanism that accounts for this effect is said to be unique to SD rats and that a similar process is not operable in Long-Evans or F344 rats. This effect does not occur in SD rats receiving lower doses atrazine and increased incidences of tumors were seen only at the high dose in the bioassays with SD rats.
4. The SD strain of rats is prone to spontaneous development of a high incidence of mammary tumors, a phenomenon that most probably is due to its unique mode of aging that does not result in ovarian failure and atrophy as occurs in women.
5. The development of mammary adenomas/fibroadenomas/carcinomas in response to endocrine disruption, even in the absences of a classical carcinogen, indicates that endogenous hormones initiate the cancer. Atrazine is a selective endocrine modulator, because it accelerates a normal process during the aging of SD rats. The fact that it does not induce tumors in male SD rats indicates that it needs a basic substrate that is intrinsic to the female SD rat. Thus, the EPA SAP on atrazine concluded that "--- there is compelling evidence to support the conclusion that the mode of action is not relevant to humans."
6. There is no evidence that atrazine is genotoxic.
7. Atrazine has no direct estrogenic activity that could account for the increased incidence of mammary tumors.
8. There are not epidemiologic data to indicate atrazine is carcinogenic to humans.
9. The EPA SAP overwhelmingly agreed that atrazine should not be classified as a "likely human carcinogen" citing "1) a positive response in only one species, strain and sex (female SD rats) with a negative response in male SD rats, F344 male and female rats and CD-1 male and female mice, 2) the mode of action for the mammary tumor response in SD rats is considered not relevant to humans, and 3) exposure levels and dose limited response makes any effects in humans unlikely. (IARC earlier, 1999, considered atrazine "not classifiable").
10. As reflected in the EPA's October 31, 2003 Interim Registration Eligibility Decision, the EPA Health Effects Division's Cancer Assessment Review Committee (CARC) concludes that the mode of action is not relevant to humans and classified atrazine as "not likely to be carcinogenic to humans."

As mentioned above, this brief list of conclusions drawn from the SAP Report and EPA's review on atrazine are consistent with my earlier review and summary of the literature on this chemical done for the USDA. A copy of that review is attached.

In summary, based on my past and present reviews of the literature on atrazine, I do not think it is a carcinogen for any species other than the female SD rat. Based on my experience as a member of Review Group One of the Report on Carcinogens, I think consideration of atrazine for listing in the Report on Carcinogens would consume valuable resources that might be more effectively directed to greater health threats. I feel strongly that such a review would reach the same conclusion reached by the EPA on atrazine, that atrazine is not a human carcinogen. Therefore, as a former member of Review Group One, I strongly recommend that the time and resources of your staff, your contractors and the various review groups involved with the Report on Carcinogens be directed to more likely carcinogens than atrazine.

Sincerely,

H. B. Matthews, Ph.D.
Consultant in Toxicology

Attachment:

Review of the "Preliminary Draft of the Hazard and Dose-Response Assessment and Characterization for Atrazine"

(page #s cited, except where noted, refer to the EPA document "Preliminary Draft Hazard and Dose-Response Assessment and Characterization)

There is no clear evidence of an association between triazine herbicides and human cancer, p. 12, Part B. However, lifetime administration of atrazine at relatively low doses, approximately 4 mg/kg, to female but not male Sprague Dawley (SD) rats, and not Fisher F344 rats or CD-1 mice resulted in early onset and increased incidences of mammary carcinomas and adenomas in 3 of 4 bioassays, p. 33 Part B. (While early onset of pituitary tumors were confirmed, increased incidences of fibroadenomas and pituitary adenomas were not observed at terminal sacrifice, p. 6.) Thus females SD rats seem uniquely sensitive to the carcinogenic effects of atrazine. Other rat strains, dogs and mice appear to tolerate significantly higher doses, p. 20, prior to developing non-carcinogenic lesions or other adverse conditions and apparently do not develop the carcinogenic lesions seen is female SD rats.

The mechanism accounting for the increased tumor incidence observed in female SD rats has been explained by eloquent research demonstrating that a neuroendocrine effect attenuates the luteinizing hormone (LH) surge necessary for normal reproductive cycling. Attenuation of the LH surge in turn disrupts the estrous cycle to result in premature reproductive aging in SD, but not Fisher rats. Since atrazine is not mutagenic and does not have direct endogenous estrogenic activity, this indirect effect is probably the most rational explanation of the carcinogenicity of atrazine in female SD rats. Support for the hypothesis of premature aging is not unanimous p. 81. At least two groups that reviewed the data on atrazine concluded that there are inconsistencies or a lack of data to support this hypothesis. It is, however, not apparent that they offered a better explanation.

Even among SD rats the adult female seems to be most sensitive. Male SD rats did not develop mammary tumors and were apparently more resistant to other adverse effects of atrazine. Also, adverse effects observed in pre- and post-natal young SD rats were observed only at higher doses. Relation of dose to effects is not discussed in detail in the summary portion of the document, but on p. 23 it appears effects in the young were observed at

doses that were higher than the LOAEL in females. Delayed vaginal opening was seen only at doses of 50 mg/kg and higher. Delayed preputial separation was observed in males at 12.5 mg/kg and higher. Similarly, the suppressed suckling induced prolactin release that resulted in lateral prostate inflammation in male offspring at 120 days was observed at doses of 50 and 100 mg/kg to the dams, p. 24. These observations would indicate that an additional 10X safety factor is not needed in the case of atrazine.

The health of developing and young humans is always a primary concern. Based on results seen in SD rats one of the primary concerns raised in this text is delayed puberty in humans. Whereas, delayed puberty is a risk factor for a number of non-cancer conditions and is not to be taken lightly; delayed puberty is not a risk factor for cancer in humans. As pointed out in the text, p. 63, just the opposite is the case. Early age of menarche is a risk factor for breast cancer.

There are numerous instances where the text states that "if" atrazine affected the hypothalamic GnRH in humans like it does in the SD rats "it is plausible to assume that a similar neuroendochrine mode of action would apply in humans". There is little evidence, however, that this is the case. In fact, the strongest evidence seems to be that, in SD rats, reproductive aging results from disruption of the LH levels thus inhibiting ovulation and retention of ovarian follicles. The ovarian follicles continue to secrete estrogen that, in turn, acts at the pituitary to cause the release of prolactin, which stimulates the breast tissue long past the normal period during which it would be stimulated. Prolonged stimulation of breast tissue results in an increased incidence of tumors. In humans, reproductive aging results from depletion of follicles from the ovary, pp. 59, 60, and a cessation of breast stimulation. If this is the case as described for SD rats in several places in the text, and as described on pp. 59, 60 for humans it would seem that atrazine could not act in the same way in humans as it does in SD rats. Further, the human condition most similar to reproductive aging in the SD rat is polycystic ovarian syndrome (PCOS). According to data cited in this report, there is little evidence that PCOS is a significant risk factor for breast cancer (Solomon, 1999) p. 61.

As indicated at several points in the text the increased incidence of tumors observed in SD rats is not attributed to a direct mutagenic effect. Therefore, as mentioned in the text, the carcinogenic effect is both most probably highly dose-dependent and non-linear. Similar non-linearity would be

expected of the prostatitis observed in males. The report states that there is no indication of an increased cancer risk as a result of prostatitis but speculates that conventional cancer testing might not detect such an outcome, p. 68. (I think conventional cancer testing would detect such an outcome if it were present.) In any case, as stated on p. 76 "The linear extrapolation is not supported by the mode of action data." That being the case it would seem that, if the human response is indeed similar to the rat, then the human response would not be expected to be linear and the low levels to which humans are exposed would be of minimal concern. (The low levels of human exposure are described in some detail in IARC Monograph on atrazine Evaluation of Carcinogenic Risks to Humans, Volume 73, p. 65, 1999. Because atrazine is degraded in treated plants there is no exposure in food. The primary source of exposure is in drinking water. Atrazine is relatively soluble in water and the maximum concentrations occur in surface water on a seasonal basis, June through July. Atrazine residues found in reservoirs at he 90^{th} percentile were about 5 ppb and rarely exceeded 20 ppb.)

In summary, increased incidences of breast tumors observed in SD rats treated with atrazine are attributed to premature reproductive aging. This mechanism action somewhat unique to the female SD rat and some other rat strains, p. 87, Part B, and is not observed in other species tested or in Fisher rats. Further, available data indicate that this mechanism of action is not relevant to humans. Therefore, increased incidences of breast tumors observed in female SD rats are, most probably, not relevant to humans. This opinion is supported by results of most reviews of the data on atrazine presented in Table 4.4 on p. 82. That is, a clear majority of the expert scientific panels that have considered all available data reached the conclusion that tumors induced by atrazine in SD rats are "not relevant" to humans.

Notes:

1. The IARC Monograph on Atrazine "Evaluation of Carcinogenic Risks to Humans", Volume 73, p. 96, 1999 states that, "A combined analysis of the results of two cohort studies of agricultural chemical production workers in the United States showed decreased mortality from cancers at all sites combined among the subset of who had had definite or probable exposure to triazine."

2. The IARC Monograph on Atrazine Evaluation of Carcinogenic Risks to Humans, Volume 73, p. 99, 1999 states that, “--- there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in Sprague-Dawley rats is not relevant to humans.”

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISKS TO HUMANS

Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances

VOLUME 73

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Carcinogenic Risks to Humans,
which met in Lyon,

13–20 October 1998

1999

A Review of the Epidemiological Studies on Atrazine and Other Chlorotriazine Herbicides

Authors

Dr. Elizabeth Delzell
Professor, Department of Epidemiology
School of Public Health
University of Alabama at Birmingham

Dr. Jack Mandel
Professor and Chairman,
Department of Epidemiology,
School of Public Health
Emory University

Dr. Charles Breckenridge
Senior Research Fellow
Human Safety Assessment
Syngenta Crop Protection, Inc.

July 19, 2004

1.0 Executive Summary

Overall, there is no basis for concluding that there is a causal association between exposure to atrazine and cancer in humans. Cohort studies conducted at the production facility over a long period of follow up have not identified any increased cancer risk, including non-Hodgkin's lymphoma cancer risk. The excess number of prostate cancer cases at the St. Gabriel facility is fully accounted for by the prostate cancer screening bias operative at the plant as a result of the advanced medical surveillance program implemented at that plant. The null results reported for prostate cancer in the large cohort of licensed pesticide applicators that are members of the Agricultural Health Study support this conclusion.

A review of the case-control studies conducted by the National Cancer Institute, principally on non-Hodgkin's lymphomas, have not established a causal association between atrazine use and the occurrence of this disease. This conclusion has also been reached in two authoritative reviews (EPA and IARC)[31,32] and by three independently published reviews (Loosli, Neuberger, Sathiakumar & Delzell).[28, 29, 4]

The ecological studies, which do not measure exposure or disease at the level of the individual, have generated null relationships, inverse relationships (Van Leeuwen, 1999)[27] and a few positive associations that have not been supported by the results from cohort studies (Mills, 1998, 2003).[8,9] The results from some of the studies have been contradictory (e.g. Kettles vs. Hopenhayn-Rich)[25, 26] or implausible (Van Leeuwen, 1999).[27]

The animal mechanistic data, reviewed elsewhere and summarized by Breckenridge et al., (2004),[30] has shown that atrazine is not genotoxic or an androgen- or estrogen-mimic. The mode of action underlying the increased incidence/earlier appearance of mammary tumors in the female Sprague-Dawley rats administered high doses of atrazine has been concluded by both EPA[31] and IARC[32] not to be relevant to humans.

2.0 Introduction

Atrazine, one of the most widely used herbicides in the U.S. today, has been manufactured and used as a broad-spectrum herbicide for approximately 45 years in the United States and throughout the world. There has been a number of toxicologic and epidemiologic studies on atrazine. An evaluation of these data, particularly those relating to human health, is important in determining if there is a causal relationship between atrazine and cancer in humans. The purpose of this review is to consider the relative strengths and weaknesses of the individual epidemiologic studies conducted on atrazine, and reach a conclusion concerning the causal association, or lack thereof, between atrazine exposure and the occurrence of cancer in manufacturing facility and agricultural workers.

Three types of studies (case-control studies, cohort (both prospective and retrospective) studies and ecologic or correlational studies) have been conducted to assess possible relationships between atrazine or other triazine herbicides and cancer in humans. These studies are summarized in sections 3 to 5, respectively. Within each section, a more detailed assessment of studies on prostate cancer and non-Hodgkin's lymphoma was conducted because there have been more studies published on these two diseases. Section 4 summarizes conclusions from published and governmental reviews of triazines.

The best studies to rely on are the cohort studies of workers exposed to triazines because these provide a better assessment of exposure than other studies and are therefore, less subject to information bias. Case-control studies are more difficult to interpret because the exposure information has been obtained mainly through a self-report of work practices or from a surrogate who provided information on a deceased subject. Thus, the potential for information bias is greater with case-controls studies. Selection bias is also a problem with case-control studies, particularly those with a fairly large proportion of people who refuse to participate. Ecological studies are the least reliable because they provide group, rather than individual, data.

3.0 Cohort Studies and Nested Case-Control Studies

3.1 Triazine Manufacturing Workers – Study Design

Sathiakumar et al. (1996)[1] conducted a cohort mortality study of approximately 5,000 workers at two plants that manufactured agricultural chemicals. The McIntosh facility located in Alabama, produced triazines from 1960 through 1982, and also produced other chemicals. The St. Gabriel facility, which is located in Louisiana, began producing triazine herbicides, mainly atrazine, in 1970, but it also has manufactured sequestrene, surfactants, and other pesticides including chlordimeform, and more recently the sulfonylurea herbicides.

Based on job title and work area, workers were categorized as having jobs involving "definite," "probable," or "possible" contact with triazines. There were 2,683 men with definite or probable exposure to triazines and 2,234 men with possible exposure. There were 74,080 person-years of follow-up and the median follow-up time was 18 years.

MacLennan et al. (2003)[2] evaluated mortality among workers at the St. Gabriel Louisiana plant with follow-up extended through 1997. Workers were classified into three groups: 1) company workers who were regular employees of the company; 2) contract production workers; and 3) contract maintenance workers. Some company workers were exposed to raw materials early in the manufacturing process rather than to atrazine or other triazines. In addition, some company workers were in supervisory or managerial jobs with little workplace chemical exposure. Contract production workers were potentially exposed to higher concentrations of triazines in the production and packaging units, but tended to be short-term workers. The exposures of contract maintenance workers varied according to specific task, but as a group their exposures were higher than company workers and lower than production workers. They tended to have longer tenure than contract production workers, but shorter tenure than company workers.

Company workers were followed from 1970 through 1997, contract production workers from 1977 through 1997, and contract maintenance workers from 1983 through 1997. The mortality rates were compared with the rates of the general population of the seven parishes of the Louisiana industrial corridor (all within 50 miles of the plant).

The cohort included 2,213 individuals, including 855 company workers, 753 contract production workers, and 605 contract maintenance workers. The cohort was 90% male. Vital status was determined for 99% of the cohort. There were 32,473 person-years of follow-up, and a median of 14.8 years of follow-up per subject.

Using the Louisiana Tumor Registry, plant medical records, and death certificates, (MacLennan et al.,2002)[3] evaluated cancer *incidence* among 2,045 workers at the St. Gabriel, Louisiana triazine manufacturing facility from 1985 through 1997. Quantitative exposure data were not available. The authors noted that unpublished data indicated that median concentrations of total dust decreased from 6.5 mg/m ^{3} in 1970 to 0.2-1.2 mg/m^3 in the 1980s as a result of engineering improvements. Workers were classified into three groups as in the mortality study (MacLennan et al.,2003)[2]. There were 757 company workers, 687 contract production workers, and 601 contract maintenance workers. The authors compared the incidence rate of the cohort to that of the population of the Louisiana industrial corridor.

There were 21,200 person-years of follow-up with a median of 12.6 years per person. The median age was 41 years. The median duration of employment was 3.8 years and the median time since first hire (latency) was 14.1 years. For males, the median time since first hire was 18 years.

In addition, the authors evaluated the potential effect of a PSA screening program on the detection of prostate cancer. PSA testing was first offered in 1989 only at the request of the plant physician. In 1992, the test was offered to all men age 50 and older and to younger men at the discretion of the plant physician. In 1994, all men age 45 and older were offered PSA testing. Men age 40-44 were offered PSA testing if they had a family history of prostate cancer or were African American.

Hessel et al. (2004)[5] conducted a case-control study nested within the cohort studied by MacLennan et al. (2002)[3], designed to explore the relationship between atrazine exposure and prostate cancer while accounting for the potential confounding effects of the screening program. Twelve cases and 130 control subjects were selected from the

original cohort. Prostate screening and occupational histories were abstracted from company records and atrazine exposures were estimated. Hire date was comparable for cases and control subjects. Nearly half of the control subjects and no cases left before the prostate-specific antigen (PSA) screening program.

3.1.1 Non-Hodgkin's Lymphoma

The results from the mortality studies, summarized for non-Hodgkin's lymphoma (NHL) in Table 1, indicate that in neither the combined analysis of the McIntosh and St. Gabriel production facilities cohort (Sathiakumar et al.,1996)[1] nor the subsequent update of the St. Gabriel facility cohort (MacLennan et al., 2003)[2] was the slight excess of NHL deaths linked to workers holding triazine manufacturing jobs for long durations of time. The results do not justify a conclusion of a causal association between triazines and NHL.

This interpretation is supported by the results of the cancer incidence study, which found that the incidence of NHL among workers was not significantly different than the incidence in the general population living in the Louisiana industrial corridor (Table 2). The number of NHL cases observed/expected in company employees, contract production workers, contract maintenance workers or all workers combined at the St. Gabriel facility was 3.0/1.0, 0/0.6, 0/0.6 and 3/2.2, respectively. None of these differences was statistically significant.

Table 1: Epidemiology: Cohort Studies – Non-Hodgkin's Lymphoma	
Reference	**Results**
Sathiakumar, 1996[1]	The mortality of 4917 subjects who worked in either of two production facilities involved with triazine manufacturing and/or packaging (McIntosh, AL; 1951 start-up and St. Gabriel, LA, 1970 start-up) for the period from 1960 until the study closing date of January 1, 1987 was evaluated. A total of five men died with NHL during the period from 1960 to 1987 (SMR =279, CI 91-552). Two of the subjects were classified as possibly having triazine-related jobs while 3 subjects definitely or probably had triazine-related jobs. Two of these 3 men worked in triazine related jobs for only 42 days prior to death making a causal association of NHL with triazine exposure unlikely.
MacLennan, 2003[2]	The mortality of 2213 subjects from the St. Gabriel production facility was tracked for the period from plant startup (1970) until 1997. There were 4 deaths observed and 1.1 death expected from NHL (SMR =372, CI =101–952); these deaths were not concentrated in the subgroup of workers with long duration of employment or many years since hire.
MacLennan, 2002[2]	Observed/Expected (SIR, 95%CI) number on NHL cases in the Syngenta employees, Contract production employees, contract maintenance employees and all employees was 3/1.0 (SIR=301;CI=62-878), 0/0.6 (SIR = 0, CI=0-615), 0/0.6 (SIR = 0, CI=0-615) and 3/2.2 (SIR = 136, CI= 28-398), respectively

Table 2

Number of Subjects (N), Person-Years (PY) and Observed/Expected Cancer Cases, SIR and 95% CI by Employee Group, Selected Forms of Cancer, LA Industrial Corridor Comparison

	Company (N = 757, PY = 6,071			Contract Production (N = 687, PY = 7,303)			Contract maintenance (N = 601, PY = 5,825)			Total (N = 2,045, PY=21,200)		
	Obs/Exp	SIR	9S%CI	Obs/Eup	SIR	95% Cr	Obs/Exp	SIR	95% CI	Obs/Exp	SIR	95% Cr
Alll cancer	27/21	129	85-167	8/9.1	88	38-173	11/10	107	53-191	48/40	114	53-152
Buccal cavity	1/1.0	101	3-561	1/0.5	210	5-1169	1/0.5	205	5-1142	3/2.0	153	32-446
Digestive system	4/3,6	106	29-270	1/1.6	65	2-3S0	4/1.8	217	59-55S	9/7.2	I25	57-238
Colorectum	**3/2.3**	130	27-381	0/O.9	0	0-424	0/1.1	0	0-342	3/4.2	71	15-206
Lung	2/3.2	62	B-223	1/1.0	96	2-536	3/1.5	197	41-577	6/5.8	104	38-225
Breast	1/0.8	119	3-665	0/0.9	0	0-429	0/0.1	0	0-4310	1/1.8	56	1-312
Prostate	8/3.7	217	94-428	1/0 8	129	3-721	2/1.9	108	13-391	11/6.3	175	87-312
Bladder	3/1.0	295	01-863	0/0.3	0	0-1308	0/0.4	0	0-889	3/1.7	175	36-511
LHC	5/2.0	253	62-591	1/1,2	82	2-459	1/1.2	82	2-459	7/4.4	159	64-328
NHL	3/1.0	301	62-078	0/0.6	0	0-615	0/0.6	0	0-615	3/2.2	136	28-398
Other cancers	3			3			0			8		

Includes Company – one malignant melanoma of the skin, one thyroid cancer and one pleural cancer, Contract production – one brain cancer, one thyroid cancer and one unspecified form of cancer.

3.1.2 Prostate Cancer

In the first mortality study (Sathiakumar et al, 1996),[1] there were no prostate cancer deaths in men with definite/probable or possible exposure to triazines (Table 3). In the most recent mortality study update for the St. Gabriel production facility (MacLennan, 2003)[2], there was one prostate cancer death compared to 0.5 expected.

Using cancer *incidence* data from among 2,045 workers at the St. Gabriel, Louisiana triazine manufacturing facility from 1985 through 1997, MacLennan et al., (2003)[2] reported 46 cancers observed and 40 expected (SIR = 114; 95% CI: 83-152). There were 11 observed prostate cancers, with 6.3 expected (SIR=175; 95% CI: 87-312). There were 9 observed and 4.9 expected prostate cancer cases among white men, 8 observed and 3.7 expected among company workers, and 6 observed and 2.0 expected among those actively employed. The increased SIR for prostate cancer was limited to men under age 60. The SIRs (95% CIs) were 336 (69-982), 261 (105-539), and 37 (1-204) for men age 49 years and younger, 50 to 59 years, and 60 years and older, respectively. The excess was similarly limited to men under age 60 when analyses were restricted to company workers. The prostate cancer increase was concentrated in company workers who were actively employed compared to inactive company workers and contract workers. Of the eleven incident prostate cancers, nine were localized at diagnosis, one was regional, and one was distant. The median age at diagnosis was 51 years.

A significantly elevated odds ratio for prostate cancer was found in the overall male cohort age 50 to 59 years and for company workers of the same age. However, the authors pointed to several observations suggesting that the increase in prostate cancer incidence was likely due to the company-based PSA testing program. The increased incidence was concentrated in the company workers who were actively employed, worked for a longer time, and therefore had more opportunities for PSA testing. The medical surveillance of the company workers was more extensive than that of the contract workers, and was more extensive than that of the general population of the industrial corridor of Louisiana. In addition, the relatively young age and early stage of the cancers at diagnosis compared with the general population of Louisiana suggested a

screening effect. Taken together, the reports of prostate cancer mortality and morbidity among triazine manufacturers do not suggest a causal role for atrazine.

Hessel et al, 2004,[5] in the case-control study nested within the cohort, found that cases had more PSA tests than control subjects (odds ratio for one or more tests = 8.54; 95% confidence interval, 1.69–82.20). There was no association between atrazine exposure and prostate cancer when those with one or more test were compared. The excess incidence of prostate cancer at St. Gabriel can be fully accounted for by the intense medical surveillance for prostate cancer at this facility.

3.2 Agricultural Workers

There are currently no peer-reviewed publications on the incidence or mortality of NHL based on cohorts of agricultural workers, The Agricultural Health Study management has scheduled a cancer incidence publication on atrazine for 2004.[6]

One cohort study has evaluated the association between triazine herbicides and prostate cancer among agricultural workers. Alavanja et al. (2003)[7] studied a cohort of 55,332 male pesticide applicators from Iowa and North Carolina. Atrazine exposure was queried on an enrollment questionnaire that asked for information on ever use, duration of use (years), frequency of use (days per year), and decade of first use. Information on an additional 49 pesticides, use of protective equipment, other agricultural exposures, lifestyle factors, family and medical history and other information were collected on the enrollment questionnaire and a take-home questionnaire.

Alavanja et al (2003)[7] did not find an association between prostate cancer incidence and atrazine (OR= 0.94; 95% CI: 0.78-1.14 for ever versus never use). The age- and family history-adjusted odds ratios for atrazine in the lowest to highest cumulative exposure score categories were: 1.0 (referent, no exposure), 1.02 (95% CI: 0.79-1.31), 0.91 (95% CI: 0.71-1.18), 0.89 (95% CI: 0.65-1.23) 0.82 (95% CI: 0.54-1.25), and 0.97 (95% CI: 0.63-1.48). The authors evaluated potential effect modification by family history for all of the pesticides in the study. The OR for atrazine and prostate cancer among men with

no family history of prostate cancer was 0.88 (95% CI: 0.72-1.09) and 1.28 (95% CI: 0.77-2.12) for men with a positive family history of the disease. The interaction was not statistically significant. Thus, there was no evidence of an increased risk of prostate cancer among pesticide applicators that reported exposure to atrazine in this study.

Table 3: Epidemiology: Cohort Studies – Prostate Cancer	
Reference	**Results**
Sathiakumar, 1996[1]	No prostate cancer deaths were observed in this study for men with definite/probable or possible exposure to triazines.
MacLennan, 2003[2]	One prostate cancer death was observed with 0.5 expected.
MacLennan, 2002[3]	In a cohort of 2,213 individuals (855 company workers, 753 contract production workers, and 605 contract maintenance workers there was one observed prostate cancer death compared to 0.5 expected. There were 11 observed prostate cancers, with 6.3 expected (SIR=175; 95% CI: 87-312). There were 9/4.9 (obs/exp) prostate cancer cases among white men, 8/3.7 among company workers, and 6/2.0 among those actively employed. The increased SIR for prostate cancer was limited to men under age 60. The SIRs (95% CIs) were 336 (69-982), 261 (105-539), and 37 (1-204) for men age 49 years and younger, 50 to 59 years, and 60 years and older, respectively.
Hessel, 2004[5]	Excess prostate cancer cases at an atrazine manufacturing facility can be accounted for by the prostate cancer screening program implemented at that facility. Cases had more PSA tests than control subjects (odds ratio for $\geq$1 test, 8.54; 95% confidence interval, 1.69–82.20). There was no association between atrazine exposure and prostate cancer when cases and controls with one or more PSA tests were compared
Alavanja, 2003[7]	There was no association between atrazine exposure and the occurrence of prostate cancer in a cohort of 55,332 male pesticide applicators from Iowa and North Carolina. (OR= 0.94; 95% CI: 0.78-1.14 for the categories ever versus never use). The age- and family history-adjusted odds ratios for atrazine in the lowest to highest cumulative exposure score categories were: 1.0 (referent, no exposure), 1.02 (95% CI: 0.79-1.31), 0.91 (95% CI: 0.71-1.18), 0.89 (95% CI: 0.65-1.23) 0.82 (95% CI: 0.54-1.25), and 0.97 (95% CI: 0.63-1.48).

There was a nested case-control study and an ecological study of the association between triazine or atrazine use and the occurrence of prostate cancer in California. Although they are included here in order to permit an overall assessment of the evidence regarding the association between atrazine use and the occurrence of prostate cancer, the study results are presented in Table 7 with the other ecological studies.

Mills (1998)[8] correlated age-adjusted cancer incidence rates (six cancer sites) for 1988-1992 with data on pesticide use (i.e., pounds of active ingredient applied annually per county) for all 58 counties in California. Among black males, the correlation coefficient for atrazine and prostate cancer was 0.67 (95% CI: 0.01-0.92); however the correlation coefficients were below zero for white, Hispanic, and Asian men. As with any ecologic study, the correlation coefficients may not represent associations that exist at the individual level because no information on either exposure to atrazine or the occurrence of cancer was obtained for individuals. Additionally, the exposure and outcome data came from the same time period; i.e., no induction time was allowed between exposure and disease onset. It was not possible to distinguish a potential effect of atrazine from other factors that might have contributed to the occurrence or detection of prostate cancer.

Mills and Yang (2003)[9] conducted a nested case-control study (222 cases, 1,110 controls) within a cohort of predominantly Hispanic members of a farm workers union in California. Prostate cancer cases were identified through computerized record linkage between the population-based California Cancer Registry and the union database for the years 1987-1999. Five age-matched controls were randomly selected for each case from the cancer-free union members who were alive through the year of diagnosis of their respective case. Information on farm employment history, including dates and locations, was available from union records. This information was linked to pesticide use reports from the Department of Pesticide Regulation in California. These reports included information on types of pesticides applied, where and when they were applied, the method of application, the size of the area treated and the amount of active ingredients applied. The number of pounds of pesticide active ingredient applied in a county in a given year was used as a measure of exposure.

Mills and Yang (2003)[9] did not report results for atrazine exposure and prostate cancer, but they reported an analysis of simazine, another triazine herbicide. After adjustment for age, date of first union membership, and duration of union affiliation, there was an elevated risk of prostate cancer with estimated high versus low exposure to simazine (OR=1.53; 95% CI: 1.02-2.28). The ORs for increasing use of simazine were 1.00

(reference), 1.52, 1.56, and 1.81 (p_{trend} = 0.03). The OR for simazine was higher for men diagnosed at a late stage (OR = 2.16; 95% CI: 1.15-4.04) versus early stage (OR = 1.20; 95% CI: 0.71-2.03).

There are limitations to this study. It did not assess the simazine exposure of individual subjects. Rather, the exposure assessment was ecological, relying on records of pesticide use by year and county and applying these to individuals based on their union work records. Exposure to pesticides was only estimated for time periods when cases and controls were union members, and the assessment was therefore likely to be incomplete. The workers were potentially exposed to multiple chemicals and these exposures were not separated in the analysis. Finally, the population under study may have been more likely than the general California population to migrate, and thus, the cases identified by the cancer registry may not have been a complete representation of all cases.

3.3 Atrazine and Prostate Cancer: Summary of the Evidence

The epidemiologic studies do not support a causal association between atrazine and prostate cancer. The correlation reported by Mills (1998)[8] was based on ecologic data and limited to black males only. Similarly, the result for simazine reported by Mills and Yang (2003)[9] was based on ecologic exposure data . Atrazine was not assessed in that study. In the Agricultural Health Study (Alavanja et al. 2003)[7], where individual data were developed for atrazine exposure, no association was found with prostate cancer.

The findings from the studies of triazine manufacturing workers also do not demonstrate a causal association between atrazine exposure and prostate cancer. There was no significant excess of prostate cancer mortality in the studies by Sathiakumar and Delzell (1996)[1] or MacLennan et al. (2003).[2] Although there was an elevated SIR for prostate cancer in the study by MacLennan et al. (2002),[3] the data strongly suggested that the apparent excess was due to the company-based prostate cancer-screening program.

The case-control study (Hessel et al, 2004)[5] nested within the cohort studied by MacLennan et al. (2002)[3] was specifically designed to explore the relationship between atrazine exposure and prostate cancer while accounting for the potential confounding effects of the screening program. There was no association between atrazine exposure

and prostate cancer when individuals with one or more PSA screening test were compared.

The results of the studies on the relationship between atrazine exposure and the occurrence of prostate cancer do not indicate a causal relationship. This conclusion is consistent with that reached by Blondell and Dellarco, (2003).[10]

"Studies of manufacturing and farming populations do not support a finding that atrazine is a likely cause of prostate cancer. The Scientific Advisory Panel stated that neither the Syngenta St. Gabriel Plant study or the Agricultural Health Study were "sufficient for EPA to conclude that there is no causal association between atrazine exposure and prostate cancer." However, the Agency does not find any results among these studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine."

4.0 Case Control Studies
4.1 Non-Hodgkin's and Other Lymphomas

The results and comments on the case-control studies on atrazine are organized by tumor type in Table 4. The relationship between triazine exposure and NHL in men was evaluated in three case-control studies[11-13] conducted in four states (Kansas, Iowa/ Minnesota, and eastern Nebraska). An additional publication pooled the results from the preceding three studies[14] and a second publication pooled the results after making a priori assumptions about pesticide groupings.[16] A fourth study evaluated the relationship between NHL and pesticide use in women in eastern Nebraska.[15]

These five studies are referred to herein as the Kansas,[11] the Iowa-Minnesota,[12] the Eastern Nebraska, [13] the pooled, [14] and the eastern Nebraska women[15] studies (Table 5). The more recent pooled study results,[16] will be described separately.

Each study included NHL cases diagnosed in the late 1970s and early 1980s (Table 5) among adult white residents (Kansas and eastern Nebraska studies: $\geq$ 21 years; Iowa-Minnesota study: $\geq$ 31 years), selected from their respective statewide cancer registries. Case ascertainment procedures were slightly different for the eastern Nebraska women study in that NHL cases were identified using ad hoc methods.

Controls were matched to each case on age and vital status. For living cases, controls up to 64 years of age were selected by random digit dialing, whereas controls 65 years and older were selected from Health Care Financing Administration records. Deceased controls were selected from state mortality files, after excluding decedents who died of lymphopoietic cancer, a malignancy of an ill-defined site, homicide or suicide. A telephone (Kansas and eastern Nebraska studies) or in-person (Iowa-Minnesota study) interview was used to ascertain exposure to agricultural chemicals. For deceased subjects, next of kin were interviewed.

The above studies shared several methodologic strengths. The cases were sampled from all incident cases in a defined population having a cancer registry that was likely to have been complete. Cases were histologically confirmed. Interviews were conducted in a presumably unbiased manner, and the participation rates were high for cases and controls. Attempts were made to corroborate subjects' reports of herbicide use. The pooled study[14] had further strengths, including enhanced precision, adjustments for potential confounding by pesticides other than triazines, and consideration of dose-response and of induction time.

The studies had major limitations as well. Exposure data were largely self-reported and therefore subject to information bias through recall errors. The percentage of subjects requiring proxy interviews varied in the studies from about 30 to 50%. Most odds ratios (ORs) were calculated using only non-farmers as the referent group. Studies of cancer and specific pesticide exposures in farmers ideally should take into account the other potential carcinogens to which farmers are exposed; hence, analyses using farmers who did not use a specific pesticide as the referent group would have been informative. With the exception of the pooled study, the investigations lacked detailed analyses by duration of exposure and time since first exposure to triazines and, further failed to control for other agricultural chemical exposures that may confound the triazine-NHL association.

The Kansas study[11] included 170 NHL cases and 948 controls. An increased odds ratio for NHL was reported for farmers who had ever used triazines, compared with non-farmers

(OR = 2.5, 95% confidence interval (CI) = 1.2-5.4) (Table 5). The study also found positive associations with phenoxyacetic acids, amides, uracils and several other herbicides and with fungicides but not with insecticides. The OR for triazine use among subjects (3 cases and 11 controls exposed) who had not used phenoxyacetic acids or uracils was not significant (OR = 2.2; 95% CI. = 0.4 – 9.1) when non-farmers were used as the referent group. The authors did not address potential confounding by other agricultural chemicals and did not describe dose-response or induction time patterns. Although the results of the study indicated a weak positive statistical association between triazines and NHL, the data are inadequate for inferring whether the excess is due to exposure to triazines per se.

The Iowa-Minnesota study[12] included 622 NHL cases and 1245 controls. Analyses controlled for potential confounding by age, vital status, state, smoking and family history of lymphopoietic cancer and several non-farming exposures and occupations. There was no significant association between triazines use and NHL (OR = l.1, 95% CI = 0.8-1.6), based on 64 cases and 133 controls that reported use, or between atrazine use and NHL (OR= 1.2; 95% CI = 0.9-1.8), based on 59 cases and 108 controls exposed. The OR was 1.3 (95% CI = 0.7-2.5) for use of atrazine before 1965 (i.e. subjects with long potential induction time). The OR for cyanazine use was 0.9 (95% CI = 0.6-1.5), based on 27 cases and 64 controls exposed. Elevated risks, on the order of 1.5-fold or greater were noted for several pesticide groups other than triazines and for individual insecticides. ORs for triazines were not, however, adjusted for exposure to other agricultural chemicals.

The eastern Nebraska study,[13] reported initially in a brief abstract, included 201 men with NHL and 725 controls. The OR was 1.4 (95% CI = 0.8-2.2) for self-reported users of atrazine compared with non-farmers. The OR for atrazine increased with duration of exposure (Ors of 0.9, 0.8, 2.0, 2.0 for 1-5, 6-15, 16-20, and 21+ years, respectively). Neither the CI's of the latter ORs nor the numbers of exposed cases and controls were cited in the abstract.[13] However, a later publication[14] provided more detailed results, described below.

The pooled study[14] incorporated data from the three case-control studies conducted in Kansas,[11] Iowa-Minnesota,[12] and eastern Nebraska[13] to evaluate the relationship between

exposure to *atrazine* in the agricultural setting and NHL among white men. The pooled data included a total of 993 cases of NHL and 2918 controls. Most analyses were adjusted for age, state, and agricultural exposures other than atrazine. Analyses pertaining to duration and other use characteristics were restricted to the data from Nebraska, the state with the most detailed information on atrazine use.

An OR of 1.4 (*95 %* CI = 1.1-1-8; 130 exposed cases, 249 exposed controls) was reported for farmers who used atrazine compared with non-farmers. Adjustments for the use of 2.4-dichlorophenoxy-acetic acid and organophosphate insecticides reduced the OR to 1.2 (*95%* CI = 0.9-1.7) for atrazine-exposed farmers compared with farmers who did not use atrazine. This decrease was most pronounced in Nebraska, where the OR dropped from 1.7 to 0.7. A two- to three-fold increase in the odds ratio was observed among long-term and frequent users of atrazine in Nebraska in analyses that did not adjust for exposure to other pesticides. When adjusted for other pesticides, however, the ORs were below unity for all duration and frequency categories except for the 21+ days per year of personal handling of atrazine. The OR of 1.4 for the latter category was based on only one exposed case and one exposed control. The OR was lower for subjects who first used atrazine on or before 1965 (i.e., for those with long potential induction time) than for subjects who first used atrazine after 1965. For example, ORs, adjusted for other pesticides, were 0.4 and 1.0 for atrazine use in or before 1965 and after 1965, respectively. Also, the OR for farmers who actually handled atrazine (OR = 1.4, 95% CI = 1.0-1,8) was similar to the OR for farmers who used atrazine but did not handle it themselves (OR = 1.6, 95% CI = 1.0-2.4). NHL.

The eastern Nebraska women study[15] included 184 NHL cases and 707 controls. Age-adjusted ORs for atrazine use on the farm and for personal handling were, respectively, 1.4 (95% CI = 0.6-3.0; 11 cases and 31 controls had used atrazine) and 2.2 (95%CI= 0.1-31.5; 1 case and 2 controls had handled atrazine).

For cyanazine use the OR was 1.3 *(*95% CI = 0.3-4.5; 4 cases and 12 controls exposed). Women who had handled organophosphates had an OR of 4.5 (95% CI= 1.1-17.9; 6 cases and 5 controls). The small numbers of subjects with specific

herbicide exposures precluded further analysis by duration or by time since first exposure to triazines. Data were not presented on ORs for atrazine use, adjusted for organophosphate use. The number of women exposed to triazines was rather small, so that OR estimates were quite imprecise. The marginally increased ORs for triazines are consistent with chance or with uncontrolled confounding by other unidentified exposures.

Table 4: Human Epidemiological, Case Control Studies - Lymphomas

Tumor Type	Reference	Results
Non-Hodgkin's Lymphoma	Hoar[11] Kansas	Elevated odds ratio were reported for a number of classes of pesticide including amides (OR = 2.9, 95% CI = 1.1 – 7.8), phenoxyacetic acid (OR= 2.2, 95% CI = 1.2 – 4.1), uracils (OR = 1.3, 95% CI = 0.7 – 2.5) and triazines (OR =2.5; 95% CI = 1.2 –5.4). No significant association between triazine use and NHL was observed when subjects who used either 2,4-D or uracil were excluded (OR = 2.2; 95% CI = 0.4 – 9.1)
	Cantor[12] Iowa/Minnesota	The odds ratio for atrazine use was not significant and close to the null value (Odds ratio = 1.2; 95% CI =0.9 to 1.8).
	Zahm [14] KS, IA-MN, NB	A pooled analysis of 3 case-control studies[14] was conducted (See Table 5). Of the 19 odds ratios calculated to test the associations between various conditions of atrazine use and NHL, 3 were statistically significant. When adjustments were made for the use of 2,4-D and organophosphate insecticides (20 tests), 2 were significant. There was no significant relationship between using triazines on the farm (OR = 1.2; 95%CI = 0.6 – 2.6) or personally handling triazines (OR = 2.2; 95% CI = 31.5) and the occurrence of NHL in 119 women.
	Zahm[15] Eastern NB Women	
	De Roos[16]	Conducted a logistic and a hierarchical logistic regression analysis of 47 pesticides use and the occurrence of prostate cancer in the 3 case-control studies evaluated by Zahm[14]. The correlation between pesticides (i.e. effect similarity) was established a priori by considering chemical class or consulting prior cancer classification decisions. Odds ratios for atrazine adjusted for other pesticide use were of borderline significant in the hierarchical regression analysis (OR = 1.5, CI = 1.0 – 2.2) and logistic regression analysis (OR = 1.6, CI = 1.1 – 2.5).
Hodgkin's Disease	Hoar[11]	Farming (type of crop, farm size, farming duration) and herbicide used was not associated with Hodgkin's disease.
Multiple Myeloma	Brown[17]	No causal association between atrazine use and multiple myeloma. (Odds ratios of 0. 8 and 1.29 were not statistically significant).
	Burmeister[18]	
Leukemia	Brown[19]	There was no significant association between atrazine use and leukemia. (OR= 1.1, 95% CI = 0.8 – 1.5)

TABLE 5
Results of Case-Control Studies of Non-Hodgkin's Lymphoma

Study location/ Triazine exposure	Number of exposed cases/controls	Unadjusted[a] OR	Unadjusted[a] 95% Cl	Adjusted[b] OR	Adjusted[b] 95% Cl
Kansas[11]					
Triazine (Any)	14/43	2.5	1.2 - 5.4		
Iowa-Minnesota[12]					
Triazine (Any)	64/133	1.1	0.8 - 1.6		
Atrazine (Any)	27/64	1.2	0.9 - 1.8		
Prior to 1965	19/32	1.3	0.7 - 2.5		
Eastern Nebraska[13]					
Atrazine (Any)	----	1.4	0.8 - 2.2		
Pooled Study[14]					
Atrazine (Any)	130/249	1.4	1.1 - 1.8	1.2	0.9 - 1.7
Handled	94/190	1.4	1.0 - 1.8		
Not handled	3.6/59	1.6	1.0 - 2.4		
Eastern Nebraska[14]	69/29	1.7	1.0 - 2.7	0.7	0.3 - 1.3
Atrazine (Any)					
Years used					
1-5 years	4/14	0.9	-	0.5	-
6-15 years	5/20	0.8	-	0.5	-
16-20 years	5/8	2.0	-	0.6	-
≥ 21 years	7/11	2.0	-	0.6	-
Annual days handled					
1-5 d/year	7/20	1.2	-	0.6	-
6-20 d/year	8/17	1.4	-	0.7	-
≥ 21 d/years	1/1	3.6	-	1.4	-
Years since first use					
Prior to 1965	10/35	1.0	-	0.4	-
After 1966	10/18	1.9	-	1.0	-
Eastern Nebraska[15] **(Women)**					
Any	11/31	1.4	0.6 - 3.0		
Personal handling	1/2	2.2	0.1 - 31.5		

a Odds ratios not adjusted for 2,4-D or organophosphate insecticide use.
b Odds ratio adjusted for 2,4-D and organophosphate insecticide use.
c Data not reported by the investigator (s)

4.2 Atrazine and NHL: Summary of the Evidence

The mortality studies (Sathiakumar et al., 1996, MacLennan, 2003)[1,2] and the cancer incidence study (MacLennan, 2002),[3] conducted in a cohort of atrazine manufacturing workers at two production facilities (McIntosh, and St. Gabriel) in the United States, do not provide evidence of an association between atrazine exposure and the occurrence of NHL that is likely to be causal. In the first mortality study (Sathiakumar et. al, 1996),[1] the cohort was large (5000 workers in two facilities), had the opportunity of daily workplace exposure to atrazine over many years, and was followed for a sufficiently long period of time to allow disease expression (74,080 person-years of exposure with a median follow-up time of 18 years). The latest update to one of the groups included in the first mortality study (MacLennan, 2003),[2] followed a subset (2234) of workers at the St. Gabriel facility through 1997 (32,473 person-years follow-up with a median of 14.8 years follow-up per worker). The cancer incidence study (MacLennan, 2002),[3] which tracked the incidence of cancer in this same cohort using the Louisiana registry, did not find an association between exposure to atrazine during manufacture and NHL. It is precisely among workers in this environment, in which there was exposure to atrazine on a daily basis over many years, and at relatively high levels, at least in the early years of plant operation, where one would expect a causal relationship to become evident. That no association has been demonstrated provides the strongest evidence that there is no causal link between atrazine exposure and NHL.

The four case-control studies conducted by the National Cancer Institute in Kansas,[11] Minnesota/Iowa,[12] and Nebraska[14,15] and reanalyzed as a pooled study[14] or using hierarchical regression analysis[16], overall have been negative with only a few borderline statistically significant odds ratios out of the many tests performed in this large dataset. These isolated significant findings could be due to chance or the inability of the investigators to control for other factors in the agricultural settings that are confounded with reports of triazine use. The authors of the pooled analysis[14] summarized the evidence of an association between atrazine use and NHL by stating.

"It has been suggested that pesticides, and herbicides in particular, may be playing a role in the large increase in the large increase in the occurrence of non-Hodgkin's lymphoma in the Midwest and other developing countries. The factors responsible must be causally associated with non-Hodgkin's lymphoma and must have an increased prevalence of exposure. Although the use of atrazine has increased over this period, the results presented in this paper, suggest that it is unlikely that atrazine exposure explains any appreciable amount of the observed increase in non-Hodgkin's lymphoma."

EPA concurred with this assessment of atrazine exposure and NHL (Blondell, & Dellarco, 2003).[10]

"The Agency does not find any results among the available studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine. EPA plans to revisit this conclusion upon receipt of new studies, especially those from NCI's Agricultural Health Study on atrazine and all cancers, prostate cancer, and non-Hodgkin's lymphoma, all of which are planned for completion in the next 1-2 years".

IARC, in their 1999 review of the NCI case control studies stated:

"A pooled analysis of the results of three population-based case-control studies of men in Kansas, eastern Nebraska and Iowa-Minnesota, United States, in which the risk for non-Hodgkin lymphoma in relation to exposure to atrazine and other herbicides on farms was evaluated, showed a significant association; however, the association was weaker when adjustment was made for reported use of phenoxyacetic acid herbicides or organophosphate insecticides. A sub-analysis of results for farmers in Nebraska, the State in which the most detailed information on atrazine use was available, showed no excess risk for non-Hodgkin lymphoma among farmers who had used atrazine for at least 15 years, after adjustment for use of other pesticides. In a case-control study of non-Hodgkin lymphoma among women in eastern Nebraska, a slight, non-significant increase in risk was seen. In all these studies, farmers tended to have an increased risk for Hodgkin lymphoma, but the excess could not be attributed to atrazine."

Neuberger, (1996)[29] concluded .

"Out of ten case/control studies published, six dealing with atrazine, none indicated any statistically significant association between atrazine and cancer. Two studies indicated marginally significant associations between triazine and cancer (OR = 1.6, 95% CI =1.0 – 2.6 and OR = 2.7, 90% CI = 1.0 – 6.9). A number of studies concerned non-Hodgkin's lymphoma, with odds ratios for atrazine or triazine exposure ranging from 1.1 to 2.2. Although positive, these were not statistically significant. At this point in time, causal criteria are not satisfied for atrazine and/or triazine(s) exposure and this malignancy."

Sathiakumar & Delzell (1997)[4] concluded.

"The relation between triazines and non-Hodgkin's lymphoma has been assessed in four independent population-based case-control studies, reporting odds ratios ranging from 1.2 to 2.5. However, chance and confounding by other agricultural exposures may have produced these weak statistical associations. Furthermore a pooled analysis of three of the case-control studies and the combined analysis of two retrospective follow-up studies did not demonstrate the types of dose-response or induction time patterns that would be expected if triazines were causal factors."

The consensus appears to be that the available evidence does not indicate that atrazine exposure is causally associated with NHL.

4.3 Other Cancers

Table 6 provides a summary of the case control studies on the relationship between atrazine exposure and other types of cancers. Only the study by Donna et al. reported results that were statistically significant at the 90th percentile confidence interval. The findings of this study has never been replicated in other study groups.

Table 6: Human Epidemiological Case Control Studies – Other Cancers

Tumor Type	Reference	Results/Comments
Colon Cancer	Hoar[20]	No statistically significant association between colon cancer and triazine exposure was found; Odds ratio for triazine use = 1.4 (95% CI 0.2 – 7.9). Comment: Reviews of studies evaluating colon cancer and farming [21, 22] have not reported
Ovarian Cancer	Donna[23,24]	In the first study[23], relative risk for ovarian tumors following exposure to general herbicide use was reported for 60 case and 127 controls to be significant (OR= 4.4, 95%CI =1.9 - 16.1). In the second study[24] of 66 cases and 126 controls increased odds ratios were observed after adjusting for age, number of live births and the use of oral contraceptives in a the definitely exposed (OR= 2.7; 90% CI = 1.0-6.9) and possibly exposed (OR= 1.8; 90% CI = 0.9 – 3.5) cases. Comment: Of the 7 "definitely exposed" individuals, only 4 actually reported using triazines while six of the 7 controls reported personal use of triazines.
Soft Tissue Sarcoma	Hoar[11]	No significant association between specific herbicide use and soft tissue sarcoma. (OR = 2.2. 95% CI = 0.4 – 9.1)

5.0 Ecological Studies

Four ecologic studies on atrazine or triazines have been reported in the literature and are summarized in Table 7. The results reported by Kettles, (1997)[25] were not supported in an update conducted by Hopenhayen-Rich (2003).[26] The results of the Mills study have been discussed extensively in Section 3.0. The study by Van Leeuwen (1999)[27] has the general limitations of most ecological studies, namely: 1) atrazine exposures was not measured at the individual subject level, but rather at the geographic regional level; 2) exposures were measured virtually concurrent with cancer occurrence; 3) factors such as duration of exposure and time since first exposure could not be analyzed; and 4) control for confounding was not adequate.

Table 7: Human Epidemiological Investigations – Ecological Studies		
Tumor Type	Reference	Results
Breast Ovarian	Kettles[25]	Results: An apparent statistical association between breast cancer incidence (Odds Ratio = 1.1 to 1.2) and drinking water of 120 Kentucky counties was reported. Comments: Possible misclassification of exposure as mentioned by the study authors, and a failure to consider population mobility, familial breast cancer history, life style difference and exposure to other confounding factors associated with drinking water and lifestyle hinder the interpretation of this study.
	Hopenhayn[26]	This study was an update to the study by Kettles. Indices of atrazine exposure (concentration in water, acres of corn planted, pounds of atrazine applied) for 120 Kentucky counties grouped into 15 districts from 1993-1997 were regressed against the incidence of breast and ovarian cancer. Null associations between breast cancer and indices of atrazine use were reported; a statistically non-significant, inverse relationship between exposure indices and ovarian cancer was also reported.
Leukemia, Prostate Testicular, Brain	Mills, 1998[8]	Positive, but not statistically significant, correlations between pounds of atrazine in Californian counties and the incidence of leukemia (r=0.40), brain (r=0.54) and testicular (r=0.41) cancers were reported for Hispanics but not for White, Blacks or Asians. For black males, a significant correlation of 0.67 was reported for prostate cancer (95% CI: 0.01-0.92). There were non-significant negative correlations between county use of atrazine and prostate cancer in White, Hispanic and Asian men.
Prostate Cancer	Mills, 2003[9]	Nested case-control study of 222, mostly Hispanic, farm workers diagnosed with prostate cancer based on the California cancer registry. 1110 age-matched controls were selected from the remaining cancer-free cohort. Exposure to simazine, was indirectly assessed for agricultural union members based upon the types of crops cultivated in a unionized agricultural area of California and the duration of time that an individual participated in the local union. The odds ratio and the 95th percentile confidence interval for low and high simazine exposure were 1.0 (1.02-2.28) and 1.53 (1.02 – 2.28) respectively. Positive associations were also reported for lindane and heptachlor with suggestive associations for dichlovos and methyl bromide.
Stomach Cancer	Van Leeuwen[27]	Stomach cancer in Ontario eco-districts was correlated with atrazine presence in water, whereas colon cancer was negatively correlated with environmental concentrations

6.0 Quotations from Regulatory Agency Reviews

6.1 EPA Interim Re-registration Eligibility Decision[31]

"The increase in prostate cancer incidence at the St. Gabriel plant in Louisiana is consistent with the intensive PSA screening. This is because prostate cancer was found primarily in active employees who received intensive PSA screening, there was no increase in advanced tumors or mortality, and proximity to atrazine manufacturing did not appear to be correlated with risk. No evidence was identified, such as dose-response evidence, that permit a determination that some of the increase was likely due to exposure to atrazine although atrazine exposure cannot be ruled out at this time as a cause."

"EPA has re-reviewed the epidemiological data regarding atrazine and cancer that were examined for the SAP meetings on atrazine in 2000 and 2003. EPA has also reviewed data that have become available since the latest meeting of the SAP in 2003. The results of those reviews are also summarized in Appendix A to this document. In brief, the Agency does not find any results among the available studies that would lead us to conclude that a potential cancer risk is likely from exposure to atrazine."

"Even though the epidemiological evidence and animal data, when viewed separately, do not support a positive cancer finding for atrazine, EPA examined the totality of animal and human data to determine if that approach showed that atrazine was likely to cause a carcinogenic response in humans. Specifically, EPA reviewed the available animal data to determine if a mechanism could be identified which supports the biological plausibility of atrazine as a human carcinogen taking into account the tumors that were identified in the epidemiological data. This review showed that (1) lymphomas, including NHL, were generally not seen in atrazine animal bioassays; (2) a mechanistic role for atrazine contributing to NHL has not been identified in laboratory studies; (3) tumors at any endocrine site other than mammary gland tumors in female SD rats (e.g., prostate, ovarian tumors) have not been identified in atrazine bioassays; (4) the SAP concluded in 2000 that the mammary gland tumors in rats caused by atrazine are produced via a mechanism not relevant to humans; and (5) the endocrine tumors that have been raised in epidemiological studies (other than mammary gland tumors) can not be biologically tied to atrazine's mode of action (i.e., decrease prolactin, decrease luteinizing hormone (LH) and suppression of ovulation). Thus, at this time, joint consideration of the available animal cancer and mode of action data and epidemiological studies, does not indicate that atrazine is likely to cause cancer in humans."

6.2 IARC, 1999 Report[32]

"A combined analysis of the results of two cohort studies of agricultural chemical prediction workers in the United States showed decreased mortality from cancers at all sites combined among the subset of workers who had had definite or probable exposure to triazine. Site-specific analyses in this subset of workers yielded no significant findings; a non-significant increase in the number of deaths from non-Hodgkin lymphoma was seen, but was based on very few observed cases."

A pooled analysis of the results of three population-based case-control studies of men in Kansas, eastern Nebraska and Iowa-Minnesota, United States, in which the risk for non-Hodgkin lymphoma in relation to exposure to atrazine and other herbicides on farms was evaluated, showed a significant association; however, the association was weaker when adjustment was made for reported use of phenoxyacetic acid herbicides or organophosphate insecticides. A sub-analysis of results for farmers in Nebraska, the State in which the most detailed information on atrazine use was available, showed no excess risk for non-Hodgkin lymphoma among farmers who had used atrazine for at least 15 years, after adjustment for use of other pesticides. In a case-control study of non-Hodgkin lymphoma among women in eastern Nebraska, a slight, non-significant increase in risk was seen. In all these studies, farmers tended to have an increased risk for Hodgkin lymphoma, but the excess could not be attributed to atrazine.

Less information was available to evaluate the association between exposure to atrazine and other cancers of the lymphatic and hemotopoietic tissues. One study of Hodgkin disease in Kansas, one study of leukemia in Iowa-Minnesota and one study of multiple melanoma from Iowa gave no indication of excess risk among persons handling triazine herbicides. In a population-based study in Italy, definite exposure to triazines was associated with a two- to threefold increase of borderline significance in the risk for ovarian cancer. The study was small, and potential confounding by exposure to other herbicides was not controlled for to the analysis.

Evaluation: There is *inadequate evidence* in humans for the carcinogenicity of atrazine. There is *sufficient evidence* in experimental animals for the carcinogenicity of atrazine.

Overall evaluation

In making its overall evaluation, (lie Working Group concluded that the mammary tumours associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism. In reaching the conclusion, the following evidence was considered:

(a) Atrazine produces mammary tumours (fibroadenomas, adenocarcinomas) only in intact female Sprague-Dawley rats (not in Fischer 344 rats, CD-I mice or ovariectomized Sprague-Dawley rats) and does not increase the incidences of other tumour types,

(b) Atrazine affects neuroendocrine pathways of the hypothalamus to accelerate the onset of reproductive senescence in female Sprague-Dawley but not Fischer 344 rats.

(c) Atrazine docs not have intrinsic oestrogenic activity

(d) There are critical interspecies differences in the hormonal changes associated with reproductive senescence.

Therefore, there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumours in Sprague-Dawley rats is not relevant to humans. Atrazine is *not classifiable as to its carcinogenicity in humans (Group 3).*

7.0 Conclusion

Overall, there is no basis for concluding that there is a causal association between exposure to atrazine and cancer in humans. Cohort studies conducted at the production facility over a long period of follow up have not identified any increased cancer risk, including non-Hodgkin's lymphoma cancer risk. The excess number of prostate cancer at the St. Gabriel facility is fully accounted for by the prostate cancer screening bias operative at the plant as a result of the advanced medical surveillance program implemented at that plant. The null results reported for prostate cancer in the large cohort of licensed pesticide applicators that are members of the Agricultural Health Study, support this conclusion.

A review of the case-control studies conducted by the National Cancer Institute, principally on non-Hodgkin's lymphomas, have not established a causal association between atrazine use and the occurrence of this disease. This conclusion has also been reached in two authoritative reviews (EPA and IARC)[31,32] and by three independently published reviews (Loosli, Neuberger, Sathiakumar & Delzell).[28, 29, 4]

The ecological studies, which do not measure exposure or disease at the level of the individual, have generated null relationships, inverse relationships (Van Leeuwen, 1999)[27] and a few positive associations that have not been supported by the results from cohort studies (Mills, 1998, 2003).[8,9] The results from some of the studies have been contradictory (e.g. Kettles vs. Hopenhayn-Rich)[25, 26] or implausible (Van Leeuwen, 1999).[27]

The animal mechanistic data, reviewed elsewhere and summarized by Breckenridge et al., (2004),[30] has shown that atrazine is not genotoxic or an androgen- or estrogen-mimic. The mode of action underlying the increased incidence/earlier appearance of mammary tumors in the female Sprague-Dawley rats administered high doses of atrazine has been concluded by both EPA[31] and IARC[32] not to be relevant to humans.

8.0 References

1. Sathiakumar, N., E. Delzell, E. & Cole, P. 1996. Mortality among workers at two triazine manufacturing plants. Am. J. Ind. Med. 29:143-151.

2. MacLennan, P. Delzell, E., Sathiakumar, N, & Meyers, S.L. Mortality among triazine herbicide manufacturing workers. Journal of Toxicology and Environmental Health, 2003, 66, 501-517.

3. MacLennan, P. Delzell, E., Sathiakumar, N, Meyers, S.L., Cheng, H., Grizzle, W., Chen, V.W., & Wu, X.C., Cancer incidence among triazine herbicide manufacturing workers. Journal of Occupational and Environmental Medicine, 2002, 44(11), 1048-1058.

4. Sathiakumar, N. & Delzell, E. 1997. A review of epidemiologic studies of triazine herbicides and cancer. Critical Reviews in Toxicology 27:599-612.

5. Hessel, P.A., Kalmes R., Smith, T.J., Lau, E., Mink, P.J., & Mandel, J**.** A nested case-control study of prostate cancer and atrazine exposure. Journal of Occupational and Environmental Medicine, 2004, 46(4), 379-385.

6. Agricultural Health Study National Advisory Panel Meeting, Bethesda MD, February 26-27, 2004.

7. Alavanja, M.C.R., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., & Blair, A. 2003, Use of agricultural pesticides and prostate cancer risk in the agricultural health study cohort. American Journal of Epidemiology, 157(9), 800-814.

8. Mills, P.K., Correlation Analysis of Pesticide Use Data and Cancer Incidence Rates in California Counties. Archives of Environmental Health, 1998, 53(6), 410-413.

9. Mills, P.K. & Yang R. 2003. Prostate cancer risk in California farm workers. Journal of Occupational and Environmental Medicine, 45(3), 249-258.

10. Blondel, J., & Dellarco, V., 2003. Review of atrazine cancer epidemiology. United States Environmental Protection Agency, OPPTS, Memorandum, October 28.

11. Hoar, S.K., A. Blair, F.F. Holmes, C.D. Boysen, R.J. Robel, R. Hoover, and J. F. Fraumeni, Jr. 1986. Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma. Journal of the American Medical Association 256:1141-1147.

12. Cantor, K.P., Blair, A., Everett,, G., Gibson, R., Burmeister, L.F., Brown, L.M., Schuman, L. & Dick, F.R., 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Research 52:2447-2455.

13. Zahm, S.H., Weisenburger, D.D., Babbitt, P.A., Saal, R.C., Cantor, K.P., & Blair A. 1988. A case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska (abstract). American Journal of Epidemiology 127:901.

14. Zahm, S.H., Weisenburger, DD, K.P. Cantor, K.P., Holmes, F.F., and Blair, A. 1993 Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma. Scand. J. Work Environ. Health 19:108-114.

15. Zahm, S.H., Weisenburger, D.D., Saal, R.C., Vaught, J.B., Babbitt, P.A., Blair, A. 1993. The role of agricultural pesticide use in the development of non-Hodgkin's lymphoma in women. Archives of Environmental Health 48:353-358.

16. De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.L., Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational Environmental Medicine, 2003, 60, 1-9

17. Brown, L.M., L.F. Burmeister, G.D. Everett, and A. Blair. 1993. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control 4:153-156.

18. Burmeister, L.F. 1990. Cancer in Iowa farmers: recent results. American Journal of Industrial Medicine, 18:295-301.

19. Brown, L.M., A. Blair, A., Gibson, R., Everett, G.D., Cantor, K.P., Schuman, L.M., Burmeister, L.F., Van Lier, S.F. & Dick., F. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Research. 50: 6585-6591.

20. Hoar, S.K., Blair, A., Holmes, F.F., Boysen, C. & Robel, R.J.. 1985. Herbicides and colon cancer. Lancet 1:1277-1278.

21. Blair, A. and Zahm, S. H. Cancer among farmers., Occup. Med. State of the Art Reviews, 6, 335-354, 1991.

22. Blair, A., Zahm,S.H., Pearce, N.E., Heinman, E.F., and Fraumeni, J.F., Jr., Clues to cancer etiology from studies of farmers. Scand. J. Work Environ. Health. 18, 209-215, 1992.

23. Donna, A., P. Betta, F. Robutti, P. Crosignani, F. Berrino, and D. Bellingeri. 1984. Ovarian mesothelial tumors and herbicides: a case-control study. Carcinogenesis 5: 941-942.

24. Donna, A., P. Crosignani, F. Robutti, P.G. Betta, R. Bocca, N. Mariani, F. Ferrario, R. Fissi, and F. Berrino. 1989. Triazine herbicides and ovarian epithelial neoplasms. Scand. J. Work Environ. Health 15:45-53.

25. Kettles, M., A., Browning, S. R., Prince, T.S., and Horstman, S.W. Triazine Herbicide Exposure and Breast Cancer Incidence: An Ecologic Study of Kentucky Counties. Environmental Health Perspectives, 105 (11), 1222-1227, 1997.

26. Hopenhayn-Rich, C. Stump, M.L., & Browning, S.R., 2002. Regional assessment of atrazine exposure and incidence of breast and ovarian cancer in Kentucky. Archives of Environmental Contaminant Toxicology, 42, 127-136.

27. Van Leeuwen, J.A, Waltner-Toews, D., Abernathy, T., Smit, B., & Shoukri, M., Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987-1991. International Journal of Epidemiology, 1999, 28, (5), 836-840.

28. Loosli, R. Epidemiology of Atrazine. 1995, Reviews of Environmental Contamination and Toxicology, 143, 47-57.

29. Neuberger, J.S. 1996. Atrazine and/or triazine herbicides exposure and cancer: an epidemiologic review. J. Agromedicine 3:9-30.

30. Breckenridge, C.B. & Stevens, J., T. A weight of the evidence evaluation of the carcinogenic potential of atrazine conducted according to USEPA's Draft Cancer Risk Assessment Guidelines. Updated, July 19, 2004.

31. USEPA, Interim Re-Registration Eligibility Decision, Case Number 0062, January 31, 2003.

32. IARC Monographs on the Carcinogenic Risk to Humans: Atrazine, 1999, Volume 73, pp 96-99.

EVALUATION OF A HORMONAL MECHANISM FOR MAMMARY TUMORIGENESIS OF THE CHLORO-S-TRIAZINE HERBICIDES: FOURTH CONSENSUS PANEL REPORT: JANUARY 13, 2000

Authors:
Prof. James Simpkins, Ph.D. (Panel Chairman)
University of Florida

Prof. Melvin E. Andersen , Ph.D.
Colorado State University

Dr. David Brusick, Ph.D., ATS
Covance Laboratories

Prof. J. Charles Eldridge, Ph.D.
Wake Forest University School of Medicine

Prof. Elizabeth Delzell, Sc.D.
University of Alabama-Birmingham

Dr. James C. Lamb, Ph.D., Esq.
Basland, Bouck & Lee, Inc

Dr. Robert F. McConnell, D.V.M., P.A.
Consulting Pathological Services

Prof. Stephen Safe, Ph.D.
Texas A& M University

Prof. Lee Tyrey, Ph.D.
Duke University Medical Center

Dr. Chris Wilkinson, Ph.D.
Jellinek, Schwartz & Connolly, Inc

SUBMITTED TO THE EPA SCIENCE ADVISORY PANEL FOR THE PUBLIC MEETING TO BE HELD JANUARY 27-28, 2000

[OPP-00637] by Novartis Crop Protection, Inc
410 Swing Road
Greensboro, N.C. 27409

1. Executive Summary

A panel of independent scientists was convened to assess the scientific evidence relevant to the mammary tumor response in female Sprague-Dawley (SD) rats exposed the chloro-s-triazines, atrazine or simazine. The panel concentrated on interpreting the results of new research recommended at prior panel meetings (6/94; 1/95; 10/96; 4/97; 5/99) with a focus on clearly delineating the mode of action for atrazine. Particular emphasis was placed on the assessment of the recent demonstration of a strong correlation between the suppression of the luteinizing hormone (LH), estrous cycle disruption, mammary gland stimulation by endogenous prolactin and estrogen and the differential occurrence of fibroadenomas and adenocarcinomas.

After comprehensive consideration of all available data, the 1999 panel reached the following consensus regarding the mode of action of mammary gland tumorigenesis by atrazine and simazine:

The scientific weight of evidence from a wide range of *in vitro* and *in vivo* studies supports the conclusion that the chloro-s-triazines (specifically atrazine and simazine) are not genotoxic and do not affect the mammary gland through a direct genotoxic mechanism. Female SD rats have a high background incidence of mammary and pituitary tumors. The ability of high doses of atrazine and simazine to induce an earlier formation of mammary and pituitary tumors in female SD rats is mediated through a non-genotoxic, threshold-based mechanism. The observation that mammary tumorigenicity is specific for the female SD rat (it does not occur in male SD rats, female or male Fischer 344 (F344) rats or in either sex of three strains of mice) further argues against any genotoxic involvement. Direct-acting genotoxic mammary tumorigens do not demonstrate such strain- and species-specificity.[1]

The formation of mammary tumors in female SD rats is due to exposure of the mammary tissue to levels of endogenous estrogen and prolactin that result from a disrupted pattern of estrous cycling characterized initially by a state of extended diestrus followed by persistent estrus. The results of *in vitro* receptor-mediated assays and *in vivo* studies show that chloro-s-triazines do not possess any intrinsic estrogenic activity, this confirming that there is not a direct estrogenic effect on the mammary tissue. Persistent diestrus and/or estrus, associated with a high incidence of mammary tumors, is characteristic of the normal pattern of reproductive senescence in the female SD rat. The panel concludes that the effects of high-dose atrazine-treatment superimposed upon the normal reproductive aging process in the female SD rat causes an earlier (than normal) occurrence of an endocrine environment favorable to the development of mammary tumors in this strain of rat. The lack of effects of atrazine on mammary tissue in ovariectomized rats provides additional support for this conclusion.

[1] Out bred strains of laboratory rats, i.e., Sprague-Dawley and Long-Evans are derived from a cross between Wistar rats and other rats including the Norway wild rat; Inbred strains such as the Fischer 344, Copenhagen, and Marshall rats were derived independently from the Wistar rat (Russell-Lindsey, 1979).

Strong scientific evidence supports the conclusion that chloro-s-triazines cause a progressive decline in the ability of the pituitary to release LH in response to estrogen in SD rats only. Blunted LH surges do occur, but fail to reach the critical level required to induce ovulation. This causes a failure in ovulation, the continued secretion of estrogen from the ovarian follicles and results in an associated state of hyperprolactinemia. The most recent research demonstrates that high doses of atrazine effectively block the estrogen-induced LH surge and disrupt estrous cycles, supporting the conclusion that chloro-s-triazines act by exacerbating the normal aging process in female SD rats. This response is not seen in other rat stains.

The site of action of chloro-s-triazines is in the hypothalamus, rather than the anterior pituitary gland. Atrazine reduces LH pulse amplitude and frequency, but does not affect pituitary responsiveness to gonadotrophin releasing hormone (GnRH). While the precise nature of the hypothalamic interaction is yet to be fully ascertained , there is a clear threshold dose of atrazine below which an effect of LH will not occur.

The panel concludes that in the SD rat model a strong statistical association has been demonstrated between parameters of estrous cycle disruption, mammary gland stimulation by endogenous estrogens and prolactin and the earlier appearance of both fibroadenomas and adenocarcinomas. Fibroadenomas are associated strongly with diestrual states and an endocrine environment of elevated prolactin with an estrogen background. Adenocarcinomas are associated strongly with estrous states and an endocrine environment of persistent elevations in estrogen and prolactin.

The breakdown of ovulatory control that characterizes normal reproductive aging in female SD rats uniquely predisposes these animals to the mammary tumorigenic effect of the chloro-s-triazine herbicides.

Therefore, the panel concludes that the atrazine-mammary tumor response in the SD model is an inappropriate surrogate for assessing human risk because reproductive senescence occurs by fundamentally different mechanisms. The SD female rat undergoes neuroendocrine failure, stays in estrus and/or diestrus and continues to produce high levels of estrogen and/or prolactin; whereas, the human female undergoes oocyte depletion accompanied by marked reduction in estrogen, but the hypothalamic–pituitary axis functions normally in the human female. In contrast to the well-described role of prolactin in the development of mammary tumors in the SD strain of rodents, there is no credible evidence of a role of prolactin in the incidence of human breast cancer.

The panel strongly emphasizes that, based on all currently available scientific information, the tumorigenic action of the chloro-s-triazines in female SD rats, is mediated through a non-genotoxic, threshold-based mechanism that is not relevant to humans. This conclusion is supported by experimental data demonstrating a clear no observable effect level (NOEL) for atrazine, not only for the formation of mammary tumors, but also for the intermediate events (estrous cycle disruption, blockage of the LH surge) leading to persistent estrus and or diestrus. As a consequence, the panel strongly concludes that risk assessment for the chloro-s-triazines should be regulated by a non-linear, margin of exposure approach.

2. Introduction

2.1. Consensus Panel Meetings/Reports

Receiving encouragement from the Environmental Protection Agency to pursue further studies to clarify the mode of action of the chloro-s-triazine herbicides atrazine and simazine, Novartis invited an independent group of scientists to thoroughly evaluate all existing data and propose additional research. Since 1994, the group has participated in a series of annual workshops and consensus-panel meetings that have resulted in the generation of a continual flow of new research information and a substantially enhanced understanding of the unique mode of action through which atrazine and simazine cause the increased incidence and/or earlier onset of mammary tumors in one sex of one strain of rats (female SD).

This document summarizes the most recent consensus of this independent group of scientists regarding the relationships between LH surge suppression, estrous cycle disruption, mammary gland stimulation by endogenous hormones and subsequent development of mammary tumors in female SD rats. It provides the basic scientific rationale for the group's conclusion that the mammary tumor response of female SD rats to high doses of atrazine is a non-genotoxic, threshold-mediated event that is not relevant to humans.

2.2 Mode of Action and Threshold Hypothesis

Several mechanistic factors have been evaluated to explain the dose, sex, species and strain specificity that characterize the chloro-s-triazine-related mammary tumors in female SD rats.

2.2.1 Genotoxic Potential

The total weight of evidence clearly indicates that atrazine and simazine are not genotoxic, since:

(i) Neither atrazine (Brusick, 1994) nor simazine (Lamb et al., 1995) show activity in a wide variety of *in vitro and in vivo* tests for genotoxicity; and

(ii) No tumorigenic effects of atrazine are observed in ovariectomized female SD rats, in male SD rats, male or female F-344 rats, or either sex of three strains of mice.

2.2.2 Estrogenic Potential

Chloro-s-triazines possess no intrinsic estrogenicity, based upon:

(i) The absence of known estrogen-mediated effects (changes in uterine weight, uterine progesterone receptors, uterine cytosolic estrogen binding and uterine DNA uptake of thymidine) following high-dose atrazine administration to ovariectomized SD rats (Tennant et al., 1994);

(ii) The lack of histopathological evidence of exogenous estrogen stimulation of vaginal and uterine tissues during 24 months of feeding of atrazine at doses which reached or exceeded the maximum tolerated dose [MTD] (McConnell, 1995);

(iii) The inability of atrazine or simazine to increase progesterone receptor levels or cell proliferation in transformed MCF-7 human breast cancer cells (Connor et al., 1996). In this same cell line, neither atrazine nor simazine (10^{-5}M) were active in inducing the transient transfection of GAL-4-ER (estrogen receptor) chimera and GAL-4 regulated luciferase reporter gene. 17β-Estradiol induced luciferase activity with an ED_{50} of 10^{-10} to 10^{-11}M.

(iv) Atrazine, simazine and cyanazine are devoid of estrogenic activity [lack of increase in uterine weight, stromal cell proliferation, epithelial cell height, progesterone or estrogen receptor, and lack of increase in the incidence of uterine fluid or estrus conversion when embedded in continuous-release pellet implants in ovariectomized female SD rats (Cook et al., 1997]. Note: Earlier positive findings using this assay were discounted when it was discovered that these pellets were contaminated with an estrogenic material (O'Connor et al., 1997).

(v) Atrazine, cyanazine, simazine and various chloro-s-triazine metabolites show no estrogenic activity in a yeast transactivation system or in a receptor competition assay at concentrations as high as 10^{-5} M (O'Connor et al., 2000, in Press).

(vi) No neoplasms or hyperplastic changes are observed in mammary tissues of ovariectomized female SD rats treated for two years with atrazine at dose levels up to and including 20.9 mg/kg/day (Morseth, 1998) that was determined to be an MTD based on decreased body weight gain and survival (Thakur, 1991). In contrast, there was a significant increase in the number of animals with mammary tumors and an earlier appearance of these tumors in intact females in the 24.4 mg/kg/day dose group (Morseth, 1998). These data show that atrazine does not possess any direct estrogenic or genotoxic activity on mammary tissues.

2.2.3 Potential Effects on Estrogen Metabolism

(i) Using data from MCF-7 cells, Bradlow et al. (1994) suggested that atrazine might increase the production of 16-α-hydroxyestrone (16-α-OHE_1) and/or reduce the production of 2-hydroxyestrone by altering the intracellular metabolism of estrogens. The 16-α-OHE_1 had been previously postulated by Davis et al. (1993) as being the type of estrogen which results in the formation of breast cancers in women. The theory is in stark contrast to work of Aldercreutz et al. (1994) which

showed through epidemiological investigations that if estrogen metabolic pathways constitutes a risk factor for breast cancer, which is at best circumstantial, the 2-hydroxyestrone is more likely a risk factor.

(ii) Safe and coworkers evaluated the effects of atrazine as well as assessing the effects of a variety of compounds that are known to inhibit or induce mammary cancer in rats on the estradiol-2-hydroxylase activity in the MCF-7 model (McDougal et al., 1997). Atrazine reduced estradiol 2-hydroxylase activity following exposure for either 2 or 48 hours. Further, no correlation between oncogenic or anti-oncogenic potential and estradiol 2-hydroxylase activity has been demonstrated; therefore, a decrease or increase in estradiol 2-hydroxylase activity does not identify mammary carcinogen potential.

(iii) The effects of several pesticides, mammary carcinogens and anti-estrogens on estradiol, 16α- and 2-hydroxylase activities and 16α-/2-hydroxylase ratios were investigated in MCF-7 cells (McDougal and Safe, 1998). Again the results indicated that in MCF-7 cells treated with these different chemicals both increased and decreased 16α-/2-metabolite ratios for each class of chemicals and the assay did not predict mammary carcinogens. A more recent report confirms that the 16α-/2-hydroxyestrone ratio is not predictive of breast cancer risk in patients (Ursin et al., 1999).

3.0 Effects of Atrazine on Reproductive Aging in Female SD Rats

3.1 The Site of Action

The ability of atrazine to inhibit the ovulatory surge of LH indicates a site of action at either the level of the hypothalamus or pituitary. Action within the hypothalamus could deprive the pituitary of the GnRH signal necessary for LH release, whereas effects within the pituitary might diminish the secretory response to that signal.

Studies conducted by Cooper et al. (1996) indicate that high doses of atrazine (75 to 300 mg/kg/day) suppressed both serum LH and prolactin surges in ovariectomized Long-Evans rats that had been pretreated with atrazine for 2 to 3 weeks. In addition, the high dose of atrazine lead to a reduction in hypothalamic norepinephrine (Cooper, 1996); therefore, a disruption of LH secretion in female SD rat is not surprising. Normal concentrations of LH and prolactin were found in the pituitary, suggesting that the hormones had not received the appropriate release signals (Cooper et al, 1995).

Daily intraperitoneal administration of atrazine to ovariectomized rats at a dose level of 200 mg/kg (but not 50 mg/kg/day) for 3 days markedly inhibited episodic pulses of LH and significantly suppressed the serum LH level relative to controls (Tyrey et al., 1996). In other experiments, the retention of pituitary responsiveness to GnRH stimulation after atrazine treatment (200 mg/kg/day x 3 days) was confirmed by the demonstration of surges in serum LH after an intravenously (iv) administered bolus of exogenous GnRH (50 ng/kg).

These results implicate the hypothalamus, rather than the pituitary as the site of action of atrazine for the inhibition of LH secretion.

3.2 The LH Surge

The estrogen-induced LH surge must reach a critical level for ovulation to occur. As female SD rats age normally, progressively decreasing amounts of LH are secreted in response to estrogen until they become insufficient to trigger ovulation. As a consequence, the ovaries continue to secrete estrogen and mammary tissue exposure to high levels of endogenous estrogen is prolonged. There now exists sound scientific evidence that high doses of atrazine disrupt the LH surge mechanism in female SD rats and accelerate the rate of naturally occurring, age-related endocrine changes specific to the female SD rat.

Characteristics of the LH surge were evaluated in SD rats fed 0, 25, 50, or 400 ppm atrazine in the diet (0, 1.8, 3.65 and 29.44 mg/kg/day, respectively) for approximately six months (Morseth, 1996b). The results clearly demonstrated that animals treated with 29.44 mg/kg/day atrazine were unable to generate a preovulatory LH surge sufficient for ovulation. Consequently, high dose atrazine treated animals enter an anovulatory state earlier than do untreated controls. This acceleration of ovulatory failure associated with atrazine results in an earlier onset of persistent estrus with more prolonged exposure to endogenous estrogen and prolactin than occurs in the control rats. The fact that no significant effects on the LH surge were observed in the low or mid-dose groups clearly supports the existence of a threshold dose.

3.3 The Estrous Cycle

Atrazine at high feeding levels alters the normal estrous cycle in female SD rats.

(i) In order to examine the effect of atrazine on the LH surge, atrazine was administered to groups of female SD rats (90/group) by gavage at dose levels of 0, 2.5, 5, 40, and 200 mg/kg/day (Morseth, 1996a). The animals were ovariectomized following 28-31 days of treatment; treatment was continued until sacrifice. Estradiol was administered subcutaneously via a surgically implanted capsule to all animals seven days after ovariectomy. All animals were bled 10 days after ovariectomy at designated intervals prior to sacrifice. 200 mg/kg/day significantly reduced the peak amplitude of the LH surge.

(ii) In a second study, high doses (75 to 300 mg/kg/day) of atrazine for 21 days disrupted estrous cycle patterns by causing periods of persistent diestrus as observed by Cooper et al. (1995), and doses of 40 to 200 mg/kg/day for 28 to 31 days caused occasional periods of extended (≥2 days) estrus (Morseth, 1996b). A dose of ≤5 mg/kg/day did not disrupt estrous cycle patterns (Morseth, 1996b), demonstrating a clear NOEL for atrazine effects on the estrous cycle and clearly indicating a threshold-based mechanism.

(iii) In a special study to examine the effects of atrazine on estrous cycle, hormone levels and tumor response (Thakur, 1991a) which showed that female SD rats fed 400 ppm of atrazine displayed an earlier appearance (after 9 months of treatment) of altered estrous cycles characterized by an increase in the percent of days in estrus. In contrast, female F-344 rats fed up to 400 ppm of atrazine (Thakur, 1991b) exhibited no effect on the percent days in estrus

(iv) In a third study, atrazine was administered to female SD rats at dietary levels of 0, 25, 50, or 400 ppm (0, 1.8, 3.65 and 29.6 mg/kg/day, respectively) for 2-years (Morseth, 1996c). Vaginal cytology was assessed on each animal daily during seven alternating 2-week intervals (i.e., weeks 1-2, 5-6, 9-10, 13-14, 17-18, 21-22, and 25-26) during the first 6 months of the study. Dr. Lee Tyrey of Duke University evaluated and classified the slides as either "diestrus," "proestrus" or "estrus." Series of days (called "blocks") of repeated diestrus or estrus that were judged to be an abnormal deviation from the regular cycling pattern in young-adult female SD rats were identified.

From weeks 2-12 (the animals were approximately 7-8 weeks of age at the start of the study), the vast majority of animals displayed normal estrous cycle patterns. There were, however, some extended periods of diestrus and sporadic blocks of $\geq$2 days in estrus, particularly in the high dose group (29.6 mg/kg/day) group. These results were consistent with those previously observed in gavage-treated SD rats in shorter-term studies (Cooper, et al., 1995, Morseth, 1996a).

By weeks 13-14, the mean percentage of days in estrus of the high dose (29.4 mg/kg/day) animals (39.6%) was statistically significantly greater than that of controls (31.4%) and most of the detected abnormalities were estrous rather than diestrus blocks. Between weeks 13-14 and the end of the study (weeks 25-26), the number of rats with abnormal patterns increased in all groups (including controls) and the abnormal cycling became more obvious particularly in the high dose group. Only 16/90 high dose animals demonstrated no abnormality of estrous cycling by weeks 25-26 (versus 42/90 controls), while 38/90 of the high dose group were in a state of persistent estrus during the entire week 25-26 observation period (versus 17/90 controls). Consequently, by weeks 25-26, animals receiving 29.4 mg/kg/day atrazine were in estrus for a significantly and substantially greater period of time (mean 62.9%) than controls (mean 46.9%).

This study clearly shows that the estrus cycle pattern most frequently associated with both normal aging and atrazine treatment in female SD rats is persistent estrus and also demonstrates the earlier appearance of persistent estrus (relative to controls) in animals fed 29.6 mg/kg/day atrazine. Since the patterns of estrous cycling in the groups fed low and mid doses of atrazine were similar to those observed in controls, the study demonstrates a clear threshold response and a NOEL of 3.65 mg/kg/day. The estrous cycle changes induced by high doses of atrazine were identical to those observed in the aging control female SD rats, with the exception that they appeared earlier.

The panel concludes that the data are entirely consistent with the concept that atrazine-induced changes in the estrous cycle are a major precursor of premature mammary tumor development in the female SD rat. The results are further confirmed by Dr. Tyrey's continued evaluation of vaginal smear data from the first 50 weeks of the ongoing 24-month study in intact vs. ovariectomized rats.

4.0 Endocrine Responsive Tumors in Female SD Rats

Through its apparent action on the hypothalamic-pituitary axis, atrazine accelerates the natural reproductive aging process and exacerbates estrous cycle disruption in female SD rats. As a consequence, high doses of atrazine can lead to profound changes in the make-up of the endogenous hormonal environment (especially estrogen and prolactin) in the female SD rat and this, in turn can result in an increased incidence and/or earlier onset of mammary fibroadenomas and adenocarcinomas, and pituitary tumors in females of this strain of rat.

4.1 Histomorphologic Characterization of Mammary Tumors

An exhaustive analysis was conducted with female SD and F344 rats to determine the relationship of atrazine exposure to estrous cyclicity and mammary gland histomorphology. Female SD and F344 rat were exposed to atrazine at dose levels of up to 400 ppm or approximately 26 to 34 mg/kg/day, respectively, for 2 years and mammary gland histomorphology was evaluated at intervals of about three months.

In female SD rats at one month, atrazine treatment was associated with a subtle change in mammary glands characterized by a tendency toward acinar development that appears to be an early indicator suggestive of increased progesterone and prolactin stimulation. The changes became more severe with increasing dose and time of treatment so that by nine month of treatment with 400 ppm of atrazine, mammary gland acinar/lobular development, secretory activity, dilated ducts with secretion and duct ectasia with galactoceles were prominent. Although the effects were first observed in treated animals, by 12 months the morphology of mammary tissue in treated and control groups was essentially the same. The data indicate that the hormonally mediated changes in mammary gland histomorphology in female SD rats exposed to high doses of atrazine are essentially identical to those seen during the normal aging process. Thus, the atrazine-mediated changes in mammary gland morphology are consistent with a primary effect on estrous cyclicity and subsequent changes in the endogenous hormone environment. The changes in mammary gland morphology are consistent with the accelerated development in female SD rats of both adenocarcinomas and fibroadenomas following high dose atrazine treatment.

In contrast to the observations in female SD rats, mammary gland histomorphology in F344 female rats showed no evidence of the effects of aging or of atrazine treatment over a period of 12 months of treatment. Clearly, the female F344 rat responds to atrazine quite differently from the female SD rat with respect to both estrous cycle response and associated mammary gland changes.

4.1.1 Role of Estrogen

The endocrine system in rodents has a profound influence on mammary tumorigenesis. Chronic exposure to estrogens has been shown to increase the incidence and cause an earlier appearance of mammary tumors in rodents (Durbin et al., 1966; Welsch, 1983; Welsch et al., 1977). As might be anticipated, ovariectomy prevents the development of mammary tumors (Durbin et al., 1966; Welsch, 1983; Welsch et al., 1977).

A time-to-tumor analysis has recently been conducted on the mammary fibroadenoma and adenocarcinoma data from the Morseth (1996c) 2-year oncogenicity study (Sielken et al., 1999). Thirty-five predictor variables including such factors as the percent of time spent in estrus or diestrus, the percent of time spent in abnormal estrus or diestrus, and whether the animals had pituitary tumors, mammary secretory activity or galactoceles were evaluated and compared to the background and the atrazine dose level using likelihood-ratio techniques. The probability that an animal would develop a fibroadenoma or an adenocarcinoma by any given time was evaluated using a multistage-Weibull time-to-tumor model. The likelihood of observing the study results if the fitted model were true was calculated for each scenario and compared to the likelihood based on the background (i.e., no dose metric was employed) or the ppm atrazine dose. The magnitude and the statistical significance of the difference between these likelihood values were calculated.

The time to response for adenocarcinomas was most strongly related to the number or percent of abnormal estrous days and moderately well related to a variety of other time measurements of days in estrus or diestrus. These results are consistent with the proposed estrogen-mediated mechanism for the earlier appearance of adenocarcinomas in female SD rats treated with high doses of atrazine.

4.1.2 Role of Prolactin

4.1.2.1 Rodents

Prolactin plays a major role in the development of spontaneous mammary tumors in rodents. All experimental treatments that chronically elevate prolactin secretion are associated with an increased incidence in mammary tumors. In rats, for example, mammary tumor incidence is increased by increasing prolactin through (i) grafting prolactin-secreting anterior pituitary glands at sites distant from the brain, (ii) administering drugs that deplete brain dopamine or antagonize pituitary dopamine receptors, or (iii) destroying the median eminence of the hypothalamus (Graves, 1990; Meites, 1972; Welsch et al., 1972 a, b). Furthermore, compounds such as ergocryptine that decrease prolactin secretion reduce the incidence of mammary tumors in rodents (Nagasawa and Morii, 1981; Quadri and Meites, 1977).

Further analysis indicates that the time to development of fibroadenomas is strongly associated with the presence or absence of mammary secretory activity, the presence or absence of the mammary galactoceles, the presence or absence of pituitary tumors, and the number of abnormal diestrual days in weeks 1 to 26. These results are consistent with the proposed mechanism for the earlier onset of mammary fibroadenomas in female SD rats exposed to high doses of atrazine (Sielken et al., 1999).

The foregoing analysis strongly supports the conclusion that the principle mechanism by which atrazine exerts its tumorigenic effect on the female SD rat mammary and pituitary glands is via a cascade of events beginning with a hypothalamic interaction and progressing through suppression of the LH surge, estrous cycle disruption and finally leading to an estrogen- and prolactin-rich tissue environment. The persistent estrus normally observed in aging in female SD rats is caused by an age-related failure of ovulation, and this predisposes untreated female SD rats to develop a high background incidence of mammary tumors and induce an earlier onset or greater incidence of spontaneously occurring mammary and pituitary tumors. It is also this characteristic aging process that places the SD female rat in a unique position with respect to its sensitivity to the chloro-s-triazine herbicides.

The impact of the low level of atrazine (in the parts-per-million range) is through effects of atrazine on the estrous cycle rather than through a direct action of atrazine on the mammary gland. The statistical support for this result is the much greater likelihood of the time-to-tumor outcomes when the dose metric in the multistage-Weibull time-to-tumor model is based on the characteristics of the hormonal disruption of the estrous cycle than when the dose metric is based on the ppm level itself. The characteristics of the hormonal disruption of the estrous cycle are much more predictive of the time-to-tumor data than the ppm level.

4.1.2.2 Humans

In contrast to the well-described role of prolactin in the development of mammary tumors in rodents, there is no credible evidence of a role of prolactin in the incidence of human breast cancer. This conclusion is based upon the absence of any relationship between levels of serum prolactin concentrations and the appearance of mammary tumors and the lack of any evidence of an increased incidence of breast cancer in long-term users of drugs that are known to increase prolactin secretion. Wang et al., (1992) failed to observe any association between serum prolactin concentrations and occurrence of mammary tumors in more than 18,000 women covering an age-range that included both pre- and post-menopausal women.

Similarly, epidemiological evidence for an association between the chronic use of drugs known to increase prolactin secretion and breast cancer has been negative. Of four classes of clinically used drugs, phenothiazines, tricyclic antidepressants, reserpine and methyldopa (Goode, et al., 1981; Kanahouma et al., 1984; Shapiro et al., 1984; Turkington, 1972), reserpine has received the most attention. Three early

reports, subsequently considered flawed, suggested that reserpine increased the risk of breast cancer (Armstrong et al., 1974; Boston Collaborative Drug Surveillance Program, 1974; Heinonen et al., 1974). A subsequent IARC study evaluated a total of 13 epidemiology studies (including the three latter ones) (IARC, 1980) and concluded that the weight of evidence showed no relationship between the use of reserpine and breast cancer. A similar conclusion was reached in what was judged to be the most rigorous study conducted (Kewitz et al., 1977) and also in a well-conducted follow-up study (Shapiro et al., 1984). Thus, no relationship between prolactin secretion-enhancing drugs and breast cancer exists in women.

4.2 Histomorphological Characterization of Pituitary Tumors

As with the mammary gland, there were no abnormal histomorphological findings in the anterior pituitary of atrazine-treated female SD rats during the first 3 months of treatment. Beginning after 9-12 months of treatment, pituitary morphology indicated the appearance of hyperplasia and adenomas that was not observed in controls. By about 18 months, however, the incidence of pituitary adenomas was similar in both atrazine-treated animals and controls. These results indicate that, as was the cases with the mammary gland tumors, the atrazine-induced pituitary tumors are an accelerated manifestation of a naturally occurring, age-related process in female SD rats. In contrast, female F344 rats showed no treatment-related histomorphological effects in the anterior pituitary during the atrazine treatment period.

4.3 Comparison of Reproductive Senescence in Female Rodents and Humans

The mode of action of atrazine for increased mammary tumors in female SD rat is an exacerbation of a premature senescence. In contrast, female F-344 rats are resistant to this effect of the herbicide. A comparison of the reproductive senescence of these two strains with that of women is important in understanding the relevance of the female SD rat to human risk assessment. There are clear and remarkable differences among estrual rodent species and menstrual humans in their etiologies for reproductive senescence. Some of the key features for these different processes are provided in Table 1. As is evident from Table 1, there is a lack of relationship between SD female rats and women for each parameter described. In addition, in contrast to the rat, human breast cancer is not promoted by prolactin nor by estrogens in the form of oral contraceptives or postmenopausal replacement. As a result of these comparisons, the panel concludes that the SD female rat is not a valid surrogate for human risk assessment for the tumorigenic effects of chloro-s-triazines.

Table 1. Comparison of Parameters of Reproductive Senescence in Female SD rats, Female F-344 Rats and Women [1]

Parameter	Sprague-Dawley Rat	Fischer-344 Rat	Women
Start of Senescence (% of normal lifespan)	30-40 %	60-70 %	60-70%
Principle cause of senescence	Hypothalamic failure to stimulate LH/FSH	Hypothalamic failure to control prolactin surges	Depletion of ovarian follicle content
LH surge capability	Lost	Maintained	Maintained
Predominant cycle pattern	Persistent estrus	Pseudopregnancy episodes	Menopause
Estrogen/progesterone ratio	Elevated/prolonged	Reduced	Reduced
Prolactin secretion	Persistently elevated	Episodically elevated	Reduced
Spontaneous mammary tumor incidence (lifetime)	30-40 %	2-5 %	8-10 %
Principal known factors that increase MT Risk	Prolactin, estrogen, chemical mutagens	Prolactin, estrogen, chemical mutagens	Family history, parity, diet, and body weight
Prolactin dependence	High	Median	None

[1]*Table after Chapin et al., 1996.*

5.0 **New Research**

5.1 A 52-Week Toxicity Study of Simazine, Atrazine and DACT Administration in the Diet to Female Rats

The panel evaluated the protocol to assess the effects of simazine, atrazine and DACT administration in the diet for 26 weeks on the estrogen-induced LH surge and the effects of treatment with the high dose of the test materials for 52 weeks on toxicity in female SD rats and to determine the concentrations associated with estrous cycle changes. The study was initiated in the Fall of 1999 and an interim report will be completed in Fall 2000.

This important study was recommended by the panel to determine if the effects of high dose atrazine on LH surges and the resulting disruption of estrous cycles and subsequent earlier appearance of mammary tumors seen in females SD rats is also seen with high doses of simazine and DACT. The study is optimally designed to assess estrous cycles throughout the treatment period, LH surges at 26 weeks prior to the appearance of mammary tumors and the subsequent association of these precursor events with mammary tumors.

5.2 Six Month Study of the Effects of Dietary Atrazine and Hydroxyatrazine on the LH Surge in Sprague-Dawley and Fischer 344 Female Rats

The panel evaluated a protocol to assess the effects of treatment with dietary atrazine with intact animals treated for 13 weeks, 26 weeks, or with the treatment beginning at 14-26 weeks on LH surge suppression (at 26 weeks) and estrous cycles in female SD rats. In addition ovariectomized SD female rats will be treated for 26 weeks with atrazine. Since atrazine is not oncogenic in F344 rats and does not disrupt estrus cycling in female F344 rats, atrazine will also be tested in F344 rats after treatment for 26 weeks to determine effects on LH surge suppression and estrous cycles. Since hydroxyatrazine is not oncogenic in female SD rats, the effects of hydroxyatrazine after 26 weeks of treatment on LH surge suppression and estrous cycles in SD rats will also be determined.

This important study was recommended by the panel: (i) to determine the effects of the major plant metabolite of atrazine, hydroxyatrazine, on LH surges and estrous cycles in the female SD rat; (ii) to compare the effects of atrazine in female F344 rats with the blunting of the LH surge and the subsequent disruption of estrous cycles seen in female SD rats; and (iii) to determine if effects in the female SD rat are seen when treatments begin from 1-13 weeks vs. 14-26 weeks compared to 26 weeks of treatment in intact and ovariectomized animals.

6.0. **Conclusions**

After complete consideration of all available data, the 1999 panel reached the following consensus regarding the mechanism of action of mammary gland tumorigenesis by atrazine and simazine.

6.1 The scientific weight of evidence from a wide range of *in vitro* and *in vivo* studies supports the conclusion that the chloro-s-triazines (specifically atrazine and simazine) are not genotoxic and do not affect the mammary gland through a direct genotoxic mechanism. The ability of high doses of atrazine and simazine to induce an earlier than normal formation of mammary tumors in female SD rats is mediated through a non-genotoxic, threshold-based mechanism. The observation that tumorigenicity is specific for the female SD rat (it does not occur in male SD rats, female or male Fischer 344 rats or in either sex of three strains of mice) further argues against any genotoxic involvement. Direct-acting genotoxic mammary tumorigens do not demonstrate such strain- and species-specificity.

6.2 The formation of mammary tumors in female SD rats is due to exposure of the mammary tissue to levels of endogenous estrogen and prolactin that result from a disrupted pattern of estrous cycling characterized initially by a state of extended diestrus followed by persistent estrus. The results of *in vitro* receptor-mediated assays and *in vivo* studies show that chloro-s-triazines do not possess any intrinsic

estrogenic activity, thus a direct estrogenic effect on the mammary tissue is not likely. Persistent diestrus and/or estrus, associated with a high incidence of mammary tumors, is characteristic of the normal pattern of reproductive senescence in the female SD rat. The panel concludes that the effects of high-dose atrazine-treatment superimposed upon the normal reproductive aging process in the female SD rat causes an earlier (than normal) occurrence of an endocrine-rich environment favorable to the development of mammary tumors. The lack of effects of atrazine on mammary tissue in ovariectomized rats provided additional support for this conclusion.

6.3 The mode of action for the chloro-s-triazines has been established with strong scientific evidence that chloro-s-triazines cause a progressive decline in the ability of the pituitary to release LH in response to estrogen. Blunted LH surges do occur, but fail to reach the critical level required inducing ovulation. This causes a failure in ovulation, the continued secretion of estrogen from the ovarian follicles and results in an associated state of hyperprolactinemia. The most recent research demonstrates that relatively high doses of atrazine effectively block the estrogen-induced LH surge and disrupt estrous cycles, clearly showing that chloro-s-triazines act by exacerbating the normal aging process in female SD rats.

6.4 The site of action of chloro-s-triazines is in the hypothalamus, rather than the anterior pituitary gland. Atrazine reduces LH pulse amplitude and frequency, but does not affect pituitary responsiveness to GnRH. While the precise nature of the hypothalamic interaction remains unknown, there is strong evidence for the existence of a clear threshold dose of atrazine below which an effect will not occur.

6.5 The panel concludes that the breakdown of ovulatory control that characterizes normal reproductive aging in female SD rats uniquely predisposes these animals to the mammary tumorigenic effect of the chloro-s-triazine herbicides. The panel considers that the mammary and pituitary tumor response in the female SD model is an invalid surrogate for assessing human risk, because this strain of rat undergoes neuroendocrine failure, stays in estrus and/or diestrus and continues to produce high levels of estrogen and/or prolactin.

6.6 A strong statistical association has been demonstrated between parameters of estrous cycle disruption, mammary gland stimulation by endogenous estrogens and prolactin and the earlier appearance of both fibroadenomas and adenocarcinomas. Fibroadenomas are associated strongly with diestrual states and an endocrine environment of elevated prolactin with an estrogen background. Adenocarcinomas are associated strongly with estrous states and an endocrine environment of persistent elevations in estrogen and prolactin.

6.7 The panel strongly emphasizes that based on all currently available scientific information, the tumorigenic action of the chloro-s-triazines in female SD rats is mediated through a non-genotoxic, threshold-based mechanism and is not relevant

to humans. This conclusion is supported by experimental data demonstrating a clear NOEL for atrazine, not only for the formation of mammary tumors, but also for the intermediate events of estrous cycle disruption and blockage of the LH surge leading to persistent estrus and or diestrus. As a consequence, the panel strongly believes that risk assessment for the chloro-s-triazines should be regulated by a non-linear, margin of exposure approach.

7.0. References

Aldercreutz, H., Gorbach, S.L., Goldin, B.R., Woods, M.N., Dwyer, J.T., and Harnalaninen, E. Estrogen Metabolism and Excretion in Oriental and Caucasian Women. J. Natl. Cancer Inst. 86:1076-1082, 1994.

Armstrong, B., Stevens, M., and Doll, R. 1974. Retrospective study of the association between use of rauwolfia derivatives and breast Cancer in English Women. Lancet 2: 673-675.

Bern, H.A., and Nandi, S. 1961. Recent studies of the Hormonal Influence in Mouse Mammary Tumorigenesis. Progr. Exp. Tumor Res. 2: 90-144.

Boston Collaborative Drug Surveillance Program. 1974. Reserpine and breast cancer. Lancet 2: 669-671.

Bradlow, H.L., D.L. Davis, G. Lin, D. Sepkovic, and R. Tiwari, The Ratio of l6a/2-Hydroxyestrone as a Biological Marker of Breast Cancer Risk. Amer. College Toxicology Symposium, Williamsburg, VA, October 1994.

Brusick, D., An Assessment of the Genetic Toxicity of Atrazine: Relevance to Human Health and Environment Effects. 1994. Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598601).

Chapin, R.E., Stevens, J.T. , Hughes, C.L. Kelse, W.R., Hess, R.A., and Daston, G.P. 1996. Symposium Overview: Endocrine Modulation of Reproduction. Fund. Appl. Toxicol. 29:1-17.

Connor, K., Howell, J., Chen, I., Liu, H., Sciarretta, K., Safe, S. and Zacharewski, T. 1996. Failure of Chloro-s-Triazine-Derived Compounds to Induce Estrogen Receptor-Mediated Responses In Vivo and In Vitro. Fund Appl. Toxicol. 30: 93-101.

Cook J.C., Van Pelt, C.S., Craven, S.C., Arnold, S.F., Obourne, J.D., Plowchalk, D.R., and O'Conner, J.C. 1997. Role of Prolactin (PRL) in Triazine-mediated Rat Mammary Tumors, The Toxicologist (36)(1), Abstract #468.

Cooper, R.L., M.B. Parrish, W.K. McElroy, G.L. Rehnberg, J.F. Hein, J.M. Goldman, T.E. Stoker and L. Tyrey, Effects of Atrazine on the Hormonal Control of the Ovary. The Toxicologist, 15(1), Abstract #1572, 1995.

Cooper, R.L., Stoker, T.E., Goldman, J.M., Hein, J., and Tyrey, G. Atrazine disrupts hypothalamic Control of Pituitary - Ovarian Function. The Toxicologist, 30(1): 66, 1996.

Davis, D.L., Bradlow, H.L., Wolff, M., Woodruff, T., Hoel, D.G., and Anton-Culver, H. Medical Hypothesis: Xenoestrogens as Preventable Causes of Breast Cancer. Environ. Health Perspectives 101:372-377, 1993.

Durbin, P.W., Williams. M.H., Jeung, N., and Arnold, J.S. Development of spontaneous mammary tumors over the life span of the female Charles River Sprague-Dawley rat: the influence of ovariectomy, thyroidectomy, and adrenalectomy. Cancer Res. 26: 400-411, 1966.

Goode, D.J., Corbett, W.T., Schey, H.M., Suh, S.H., Woodie, B., Morris, D.L., and Morissey, L. Breast cancer in hospitalized psychiatric patents. Am. J. Psychiat. 138: 804-806, 1981.

Greaves, P., Factors affecting development and growth of mammary neoplasma. In *Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation,* Elsevier Press, Amsterdam, pp. 64-69, 1990.

Gullino, P.M., Pettigrew, H.M., and Grantham, F.H. N-nitrosomethylurea as a mammary gland carcinogen in rats. J. Nat'l. Canc. Inst. 54:401-414, 1975.

Heinonen, O.P., Shapiro, S., Tuominen, L., and Tarunen, M.I. Reserpine use in relation to breast cancer. Lancet 2: 675-677, 1974.

International Agency for Research on Cancer (IARC). Monographs on the evaluation of the carcinogenic risk of chemicals to humans. pp. 213-241, 1980.

Kanahouma, S., Gowdy, J.M., and Solomon, J.D. Phenothiazines and breast cancer. J. Natl. Med. Assoc. 76: 785-788, 1984.

Kewitz, H., Jesdinsky, H.J., Schroter, P.W., and Lindtner, E. Reserpine and breast cancer in women in Germany. Eur. J. Clin. Pharmacol. 11: 79-83, 1977.

Lamb, J. et al. Weight of the Evidence on the Oncogenic Potential of Simazine: Consensus Panel Report. March 21, 1995. EPA MRID No. 43598640.

Lindsey, J.R. Historical Foundations. Chapter 1. In The Laboratory Rat. Volume 1. Biology and Diseases. H.J. Baker, J.R. Lindsey, and S.H. Weisbroth, Eds. American College of Laboratory Animal Medicine, Academic Press, N.Y., pp. 1-36.

McConnell, R.F. A histomorphologic reevaluation of the ovaries, uterus, vagina, mammary gland, and pituitary gland from Spraque-Dawley and Fischer 344 female rats treated with atrazine. MRID Report # 43598622, 1995.

McDougal, A., Wilson, C., and Safe, S. Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cell by pesticides and carcinogens. Environ. Toxicol. Pharmacol. 3: 195-199, 1997.

McDougal, A., and Safe, S. Induction of 16alpha-/2-hydroxyestrone metabolite ratios in MCF-7 cells by pesticides, carcinogens, and antiestrogens does not predict mammary carcinogens. Environ. Health Perspect. 106: 203-206, 1998

Meites, J., Regulation of prolactin and estrogen to mammary tumorigenesis in the rat. J. Natl. Cancer Inst. 48: 1217-1224, 1972.

Morseth, S. L. Evaluation of the LH Surge in Atrazine-Exposed Female Sprague-Dawley Rats - 28-Day Study. 1996a. EPA MRID No. 43934406.

Morseth, S. L. Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats - 6 Month Report (Study No. CHV 2386 111). 1996b. 2 Volumes. MRID No. 44152102.

Morseth, S. L. Chronic (12/24 Month) Study in Rats with Atrazine Technical (Study No. CHV 2386-108) 1996c. 2 Volumes. MRID No. 44152104.

Nagasawa, H., and Morii, S. Prophylaxis of spontaneous mammary tumors by temporal inhibition of prolactin secretion in rats at young ages. Cancer Res. 41: 1935-1937, 1981.

O'Connor, J.C., Cook, J.C., Van Pelt, C.S., Arnold, S.F., and O'Bourne, J.D. Weak Estrogenic Activity from Continuous Release Pellets. Reprod. Toxicol. 11(1): 1-13, 1997.

O'Connor, J.C., Plowchalk, D. R., Van Pelt, C. S., Davis, L. G., Cook, J. C. Role of Prolactin (PRL) in chloro-s-triazine-medicated rat mammary tumors. J. Toxicol. Environ. Health, In Press, 2000.

Quadri, S.K., and Meites, J. Regression of spontaneous mammary tumors in rats by ergot drugs. Proc. Soc. Exp. Biol. Med. 138: 999-1001, 1977.

Shapiro, S., Parsells, J.L., Rosenberg, L., Kaufman, D.W., Stolley, P.D., and Schottenfield, D. Risk of breast cancer in relation to the use of rauwolfia alkaloids. Eur. J. Clin. Pharmacol. 26: 143-146, 1984.

Sielken, R.L., Valdez-Flores, C., and Holden, L. Palpable Tumors in Sprague-Dawley Rats: Time-to-Tumor Analysis. Report Date: October 27, 1999. Submitted to the EPA on October 29, 1999.

Simpkins, J. W. et al. Evaluation of a Hormonal Mechanism for Mammary Tumorigenesis of the Chloro-s-triazine Herbicides: Consensus Panel Report. 1995 EPA MRID No. 43598620.

Simpkins, J. W. et al. Evaluation of a Hormonal Mechanism for Mammary Tumorigenesis of the Chloro-s-triazine Herbicides: Second Consensus Panel Report. 1996 EPA MRID No. 44152104.

Simpkins, J. W. et al. Evaluation of a Hormonal Mechanism for Mammary Tumorigenesis of the Chloro-s-triazine Herbicides: Third Consensus Panel Report. 1997 EPA MRID No. 44315401.

Tennant, M.K., Hill, D.S., Eldridge, J.C., Breckenridge, C.B., Stevens, J.T. Chloro-s-triazine antagonism of estrogen action: limited interaction with estrogen receptor binding. J. Toxicol. Environ. Health 43: 197-211, 1994.

Thakur, A.K., Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine Technical. Hazleton Washington Report 483-278. 1991a. EPA MRID No. 42085001.

Thakur, A.K., Determination of Hormone Levels in Fisher-344 Rats Treated with Atrazine Technical. Hazleton Washington Report 483-279. 1991b. EPA MRID No. 42085002.

Tsubura, A. L., Isuno, Y., Shoji, T., Kusunose, N., Morii, S. Influence of strain and sex on local development of mammary tumors induced by direct application of DMBA powder to rat mammary glands. Acta Pathol. Jpn 409-13, 1990.

Turkington, R.W. Prolactin secretion in patents treated with various drugs. Phenothiazines, tricyclic antidepressants, reserpine, and methyldopa. Arch. Int. Med. 180: 349-354, 1972.

Tyrey, L., Stoker, T., Hein, J. and Cooper, R. Atrazine suppression of luteinizing hormone secretion in the rat. Program of the U.S. Environmental Protection Agency Symposium on Susceptibility and Risk, Durham, 1996.

Ursin, G., London, S., Stanczyk, F.G., Gentzschein, E., Pagannini-Hill, A., Ross, R.K., and Pike, M.C. 1999. Urinary 2-Hydroxyestrone/16α-Hydroxyestrone Ratio and Risk of Breast Cancer in Postmenopausal Women. J. Nat. Cancer Institute 91(12): 1067-1072.

Welsch, C.W., Hormones and murine mammary tumorigenesis: an historical perspective. In: Hormonal Regulation of Experimental Mammary Tumors, B. S. Leung. Ed., Eden Press, Montreal. pp. 1-29, 1983.

Welsch, C.W., Adams, C., Lambrecht, L.K., Brooks, C.K., 17 β–estradiol and enviod mammary tumorigenesis in C3H/HeJ female mice: counteraction by concurrent 2-bromo-α-ergocriptine. Br. J. Cancer 35: 322-328, 1977.

Welsch, C.W., Jenkins, T.W., Meites, J. Increased incidence of spontaneous mammary tumors in the female rat grafted with multiple pituitaries. Cancer Res. 30: 1024-1029, 1972a.

Welsch, C.W., Nagasawa, H., Meites, J. Increased incidence of spontaneous mammary tumors in the female rats with induced hypothalamic lesions. Cancer Res. 30: 2310-2313, 1972b.

Wang, D.Y., DeStavola, B.L., Bulbrook, R.D., Allen, D.S., Kwa, H.G., Fentimen, I.S., Hatward, J.L., and Millis, R.R. Relationship of blood prolactin levels and the risk of subsequent breast cancer. Int. J. Epidemiol. 21: 214-221, 1992.

Yoshida, H., Suzuki, M., Okugawa, K., Wada, S., Fukunishi, R., Okamoto, S., and Matsumoto, K. Mammary carcinoma induced by a series of intragastric intubations of 7,12-dimethylbenz(a)anthracene in gonadectomized female and male Sprague-Dawley rats. Gann 73:539-542 (1982).

A Weight of the Evidence Evaluation of the Carcinogenic Potential of Atrazine Conducted According to USEPA's Draft Cancer Risk Assessment Guidelines

Authors

Charles Breckenridge & Jim Stevens

Updated
July 19, 2004

Syngenta Crop Protection, Inc.
Post Office Box 18300
Greensboro, NC 27419

TABLE OF CONTENTS

1.0 Executive Summary

The mode of action underlying the mammary tumor response observed in female Sprague-Dawley (SD) rats administered high doses of atrazine for a lifetime has been evaluated based upon procedures outlined in EPA's draft cancer risk assessment guidelines. Genotoxicity has been ruled out as a possible mode of action using a weight-of-evidence approach. Tier 1 and Tier 2 level tests of estrogenic potential have been conducted and have shown that atrazine is not estrogenic. In epidemiological studies of workers in atrazine production facilities and of agricultural workers, no causal association between exposure to atrazine and any disease has been found.

In evaluating the effects of atrazine on mammary tumor development in the female SD rat, an experimental basis has been established for a cascade of endocrine-related changes beginning with luteinizing hormone (LH) surge suppression, followed by estrous cycle disruption and leading to an earlier appearance and/or a higher incidence of fibroadenomas and adenocarcinomas. This pattern of endocrinologic aging has been extensively described for the female SD rat. High doses of atrazine accelerate the normal reproductive aging process in this strain of rat.

Furthermore, the response observed in the female SD rat is unique to this strain of rat, since neither the Fisher-344 rat nor 3 strains of mice have demonstrated any tumorigenic effect in lifetime bioassays (Stevens et. al., 1999). In addition, low doses of atrazine, even in the highly sensitive SD rat, have no effect on LH, estrous cycle disruption or mammary tumor development. The lack of a tumorigenic response at low doses of atrazine (~ 3.5 mg/kg/day) is due to the operation of a physiologically-based threshold. High doses of atrazine suppress LH hormone release such that there is an insufficient titer of LH in the blood to trigger ovulation. When ovulation fails, follicles within the ovum continue to produce estrogen for another day until another ovulatory LH surge occurs. Repetitive failure of ovulation creates a state of persistent estrus resulting in the hyperstimulation of the mammary gland by estrogen and prolactin.

In chronic bioassays on natural and synthetic estrogens, it has been established that prolonged stimulation of the mammary gland with estrogen lead to development of adenocarcinomas. In contrast, high-level stimulation of the mammary gland with prolactin has been shown to be linked to the development of fibroadenoma as a result of ductal enlargement, lobulo-alveolar development, the development of secretory activity and the formation of milk cysts (galactoceles).

In summary, a detailed, comprehensive and experimentally supported description of the mode of action underlying the mammary tumor response observed in SD rats at high doses of atrazine has been presented. This is supported by a rich research literature on endocrinological aging in various rodent species, and an extensive histological description of the effects of various hormonally active drugs including oral contraceptives.

Furthermore, a panel of experts including endocrinologists, pathologists, epidemiologists, molecular biologists and toxicologists have assisted in the guidance of the research on the mode of action. The consensus opinion of this group has been and firmly remains that all available evidence supports the following common mechanism of action that is only triggered at high doses of atrazine:

- The mammary tumor response in atrazine-treated female SD rats is mediated by a non-genotoxic, threshold-based mechanism leading to LH suppression, failed ovulation, and estrous-cycle disruption.

- The mammary tumor response in atrazine-treated female SD rats occurs as a result of estrous-cycle disruption, which leads to an endocrine environment (prolonged exposure to endogenous estrogen associated with extended estrus) favorable to mammary tumor formation.

- A likely site of action of atrazine is the hypothalamus, since the pituitary LH response to exogenously administered gonadotrophic releasing hormone (GnRH) was restored after high dose atrazine treatment if GnRH was administered.

- By inhibiting the LH surge and subsequent ovulation, atrazine exacerbates a condition to which the SD rat is normally predisposed and which is, in fact, the normal cause of reproductive senescence in this strain.

- The available data establish a causal relationship between atrazine exposure, an altered hormonal environment (resulting from estrous-cycle disruption), and the occurrence of mammary tumors in the SD rat.

- Data support a no-observed-effect-level (NOEL) for atrazine effects on mammary tumor development or endocrine effects at a feeding level $\leq$ 70 ppm.

Based on a total weight-of-evidence, the expert panel concluded that the female Sprague-Dawley rat is a poor surrogate for assessing mammary tumor risk in the human female as reproductive senescence in these two species occurs by a fundamentally distinctly different mechanism; i.e., neuroendocrine failure versus oocyte depletion, respectively. Atrazine promotes an acceleration of reproductive aging that occurs spontaneously in the female SD rat, i.e., central nervous system failure with age. The effect of atrazine superimposed on normal aging events together result in an earlier than normal ovulatory failure. Hence, the panel concluded that the mammary tumor response in rats to high doses of atrazine in female SD, is a non-genotoxic, threshold-mediated event that is not relevant to humans.

In accordance with the EPA's proposed guidelines for carcinogen risk assessment, the overwhelming weight of the evidence indicates that the appropriate classification for atrazine is "Not Likely to be Carcinogenic to Humans". This classification is consistent with the total weight of scientific evidence as well as recent reviews by IARC,1999 and the National Registration Authority of Australia, 1997.

2.0 Objectives

The USEPA Proposed Guidelines for Carcinogen Risk Assessment[1], which were issued as a draft in April, 1996, have been the topic of numerous Science Advisory Board meetings from 1997 to 2003. Although the guidelines are still not finalized, they were used to conduct a weight of the evidence assessment of the carcinogenic potential of pesticides regulated under FIFRA.

The purpose of this document is to evaluate the carcinogenic potential of atrazine in humans according to this guidance by:

1) Summarizing the animal evidence factors;

2) Summarizing the human evidence factors (See Delzell et al., 2004);

3) Evaluating alternative modes of actions;

4) Identifying key events underlying the carcinogenic response in animal bioassays;

5) Summarizing why the final proposed mode of action is the most plausible;

6) Evaluating the range of doses over which the dose-response remains linear;

7) Evaluating whether the carcinogenic response observed in animals is relevant to man and selecting an appropriate benchmark to regulate human exposure;

8) Evaluating whether the endocrine response observed in animal bioassays is relevant to humans and selecting an appropriate toxicity endpoint for regulating human exposure.

3.0 Hazard Identification: Animal Evidence Factors

3.1 Carcinogenic Response in the Female Sprague-Dawley Rat

High doses of atrazine cause an increased incidence and/or earlier appearance of spontaneously occurring mammary tumors in the female Sprague-Dawley rat. This response (Figure 1) was first clearly demonstrated in a 1986 study conducted by American Biogenics Corporation (ABC)[2]. The response, although highly variable, has been confirmed in subsequent studies conducted at Hazleton[3,4] (Figure 2).

Figure 1: Kaplan-Meier Estimates of Mammary Tumor Incidence in Atrazine-Treated Sprague-Dawley Rats (American Biogenics Study 410-1102)

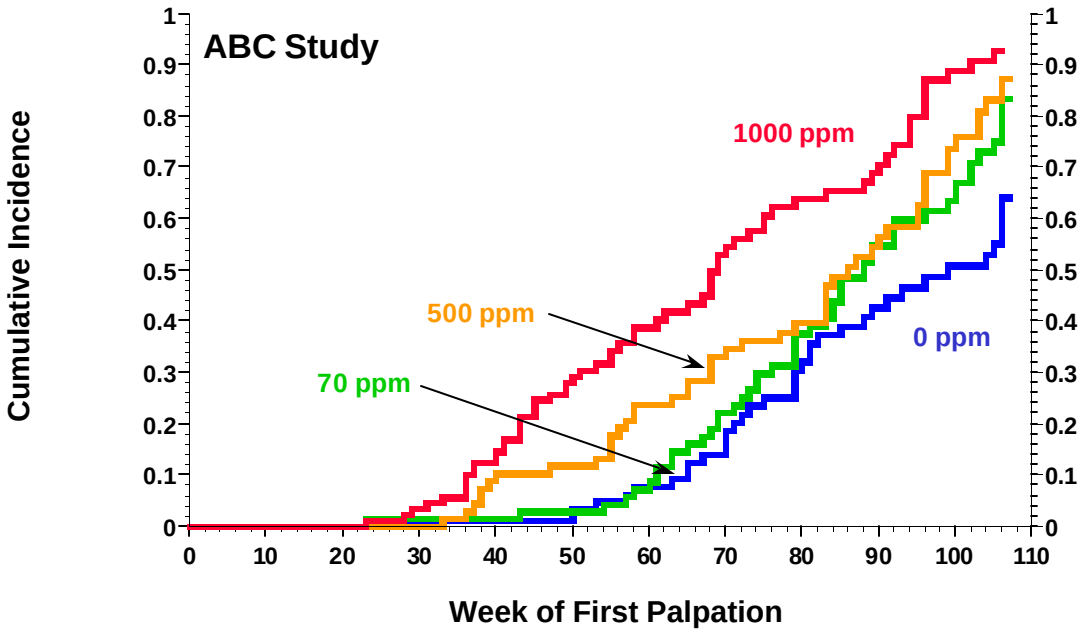
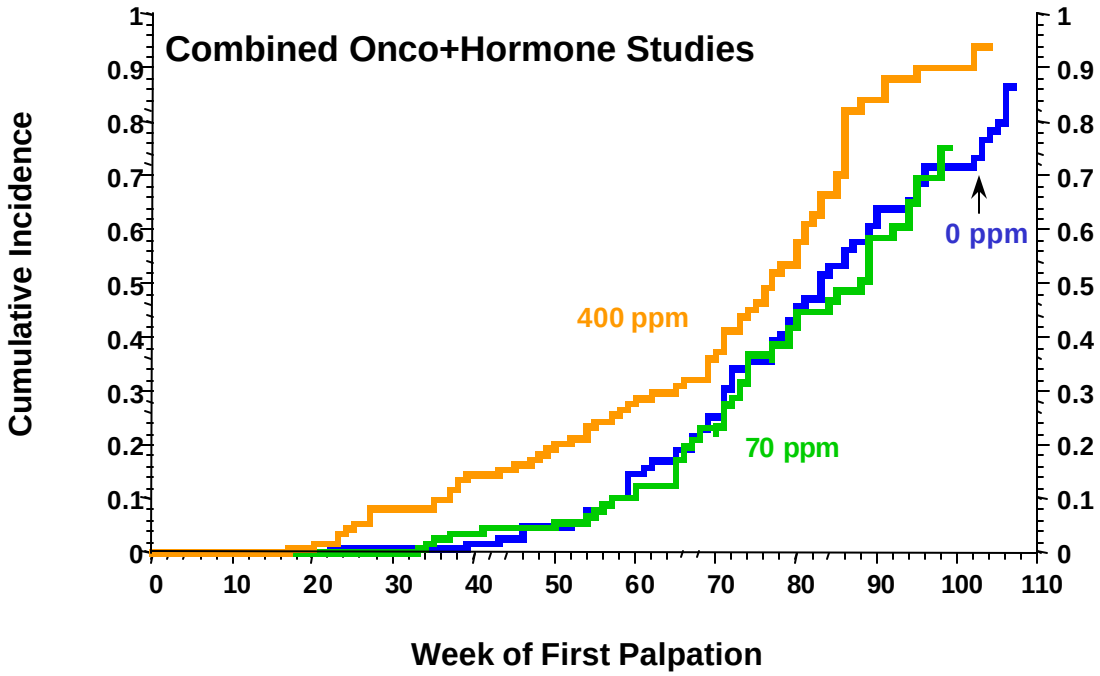
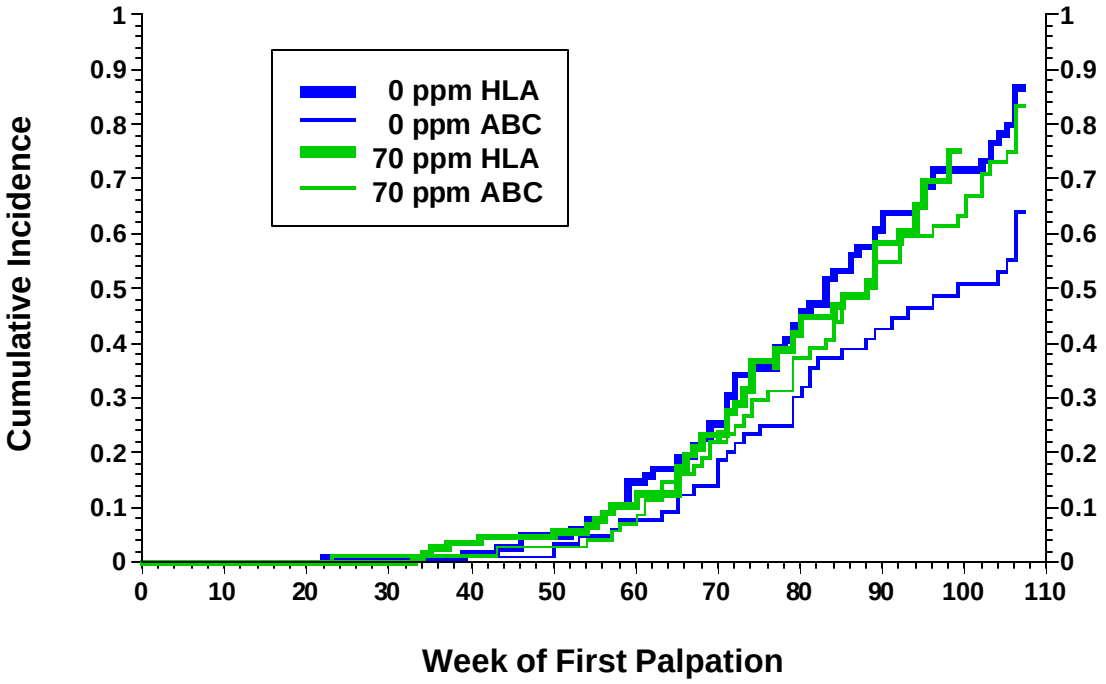


Figure 2: Kaplan-Meier Estimates of Mammary Tumor Incidence in Atrazine-Treated Sprague-Dawley Rats (HLA Studies 483-275; 483-278)



The no observable effect level (NOEL) for mammary tumors has been established experimentally at a dietary level of ≤ 70 ppm (~ 3.5 mg/kg/day). The differences in NOELs between studies is due to the high variability in the spontaneous occurrence of mammary tumors in the female Sprague-Dawley rat. This is evident in Figure 3, which provides a comparison of Kaplan-Meier plots of the cumulative mammary tumor incidence for the control and 70 ppm feeding levels from the ABC and Hazleton studies. The statistically significant increase and earlier onset observed in the 70 ppm group of the ABC study compared to the concurrent control are likely due to the lower than normal control incidence observed in this study. The Kaplan-Meier plots of the data for the 70 ppm group from the Hazleton study overlie the comparable plots from the ABC study and the Hazleton concurrent control group.

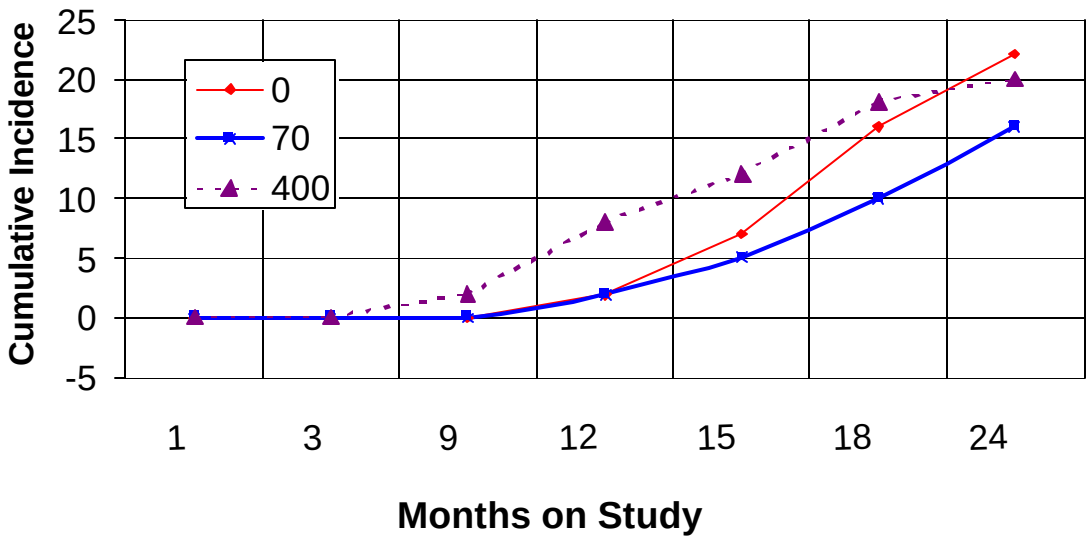
Figure 3: Comparison of the Kaplan-Meier Plots for the Control and 70 ppm Groups from the American Biogenics and Hazleton Studies



3.2 Site-Specificity of the Carcinogenic Response in Female Sprague-Dawley Rats

Aside from the mammary gland, there has been no consistent association between atrazine treatment and the incidence of any other tumor type in bioassays with female Sprague-Dawley rats. In a serial sacrifice study conducted by Hazleton[4], pituitary tumors occurred earlier in the 400 ppm atrazine group, but not in the 70 ppm group, compared to the untreated control group (Figure 4). There was no effect of treatment on pituitary tumor incidences in the 400 ppm atrazine group after 24 months.

Figure 4: Incidence of Pituitary Tumors in Female Sprague-Dawley Rats from the Hazleton Serial Sacrifice Study (HWA Study No. 483-278)

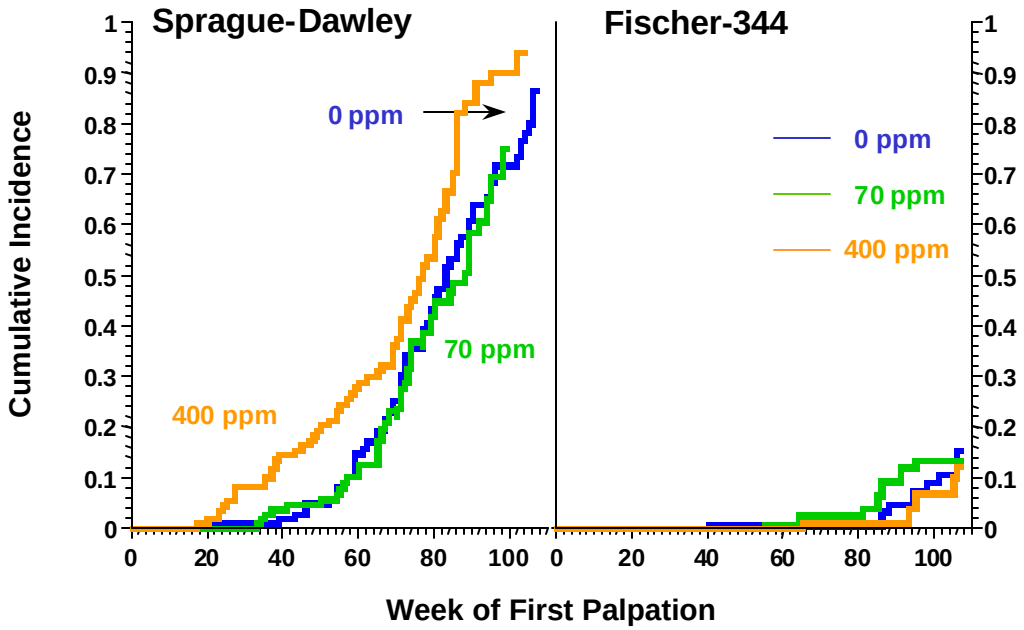


3.3 Sex- and Strain-Specificity of the Carcinogenic Response in Rats

The tumorigenic response in rodent bioassays was limited to one sex of one strain, the female Sprague-Dawley rat. Atrazine had no carcinogenic effect in the male Sprague-Dawley rat[2]. A 2-year feeding study conducted by Hazleton on atrazine in male and female Fischer-344 rats[5,6] indicated that atrazine was not carcinogenic in either the male or the female Fischer-344 rat at a maximum tolerated feeding level of 400 ppm. The carcinogenic response reported by Pinter et al. in the male mammary gland of the Lati strain of the Fischer–344 rat[7] has been discounted because of limitations in experimental design and conduct.[8] EPA has also noted the limitations of the Pinter study.[9]

Atrazine did not have any effect on mammary tumor incidence or onset in the female Fischer-344 rat. Furthermore, the untreated Fischer-344 female had a very low spontaneous incidence of mammary tumors compared to a high spontaneous incidence found in the concurrent study conducted with the Sprague-Dawley rat (Figure 5).

Figure 5: Kaplan-Meier Plots of the Cumulative Mammary Tumor Incidence in Female Sprague-Dawley and Fischer-344 Rats Treated with Atrazine[3,4,5,6]



3.4 Species-Specificity of the Carcinogenic Response

The carcinogenic potential of atrazine has been investigated in a number of studies on different strains of rats[2-6, 10-13] and mice[14-16]. The results, which are summarized in Table 1, have been highly variable for mammary tumors in female Sprague-Dawley rats, and uniformly negative for the male Sprague-Dawley, male and female Fischer-344 rat, and the male and female mouse.

Table 1: Summary of Carcinogenicity Studies on Atrazine Conducted by Novartis

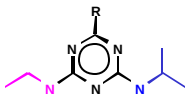
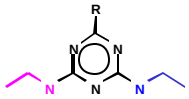
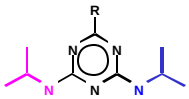
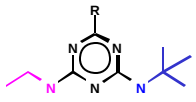
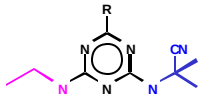
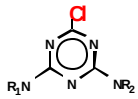
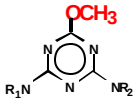
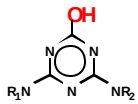
Study Reference	Species	Strain	Sex	Levels (ppm)	Results
Mayhew [2]	Rat	Sprague-Dawley	M & F	0, 10, 70 500, 1000	Male: Negative Female: Positive (70, 500, 1000 ppm)
Thakur [3]	Rat	Sprague-Dawley	F	0, 70, 400	Positive: (Early Onset) at 400 ppm
Thakur [4]	Rat	Sprague-Dawley	F	0, 70, 400	Positive: (Early Onset) at 400 ppm
Morseth [10]	Rat	Sprague-Dawley	F Intact	0, 25, 50, 70, 400	Positive: (Early Onset) at 400 ppm
Morseth [10]	Rat	Sprague-Dawley	F Ovex	0, 25, 50, 70, 400	Negative
Spindler & Sumner [11]	Rat	Sprague-Dawley	M & F	0, 10, 100, 1000	Male: Negative Female: Positive (10, 1000 ppm)
Rudzki [12]	Rat	Sprague-Dawley	F	0, 10, 50, 500	Negative
Turnier & Pettersen [13]	Rat	Sprague-Dawley	F	0, 15, 30, 50, 70, 400	Positive: (Early Onset) at 400 ppm
Thakur [6]	Rat	Fischer-344	F	0, 10, 70, 200, 400	Negative
Thakur 5	Rat	Fischer-344	M & F	0, 10, 70, 200, 400	Negative
Sumner [14]	Mouse	CD-1	M & F	0, 10, 300, 1000	Negative
Hazelette & Green [15]	Mouse	CD-1	M & F	0, 10, 300, 1500, 3000	Negative

3.5 Structure-Activity Relationships

In considering the weight of the evidence regarding the carcinogenic potential of a chemical, it is important to determine whether structurally similar chemicals cause similar tumor responses in chronic bioassays. An evaluation of structure-activity relationships (SAR) assist in identifying the active moiety, as well as to provide insight into the mode of action of a group of structurally related chemicals.

Several structural analogs and/or metabolites of atrazine have been evaluated for carcinogenic potential in chronic bioassays in female Sprague-Dawley rats. The results summarized in Figure 6, indicate that high doses of the chloro-*s*-triazines (atrazine, simazine, propazine, terbuthylazine and cyanazine) have been consistently associated with the earlier appearance or increased incidence of mammary tumors in the female Sprague-Dawley rat.[17-18] The S-methyl triazines evaluated (ametryn,[19] prometryn[20] and terbutryn;[21]) were negative. For the methoxy-*s*-triazines, prometon[22] was negative, but terbumeton[23] was positive. Hydroxyatrazine, which is a member of a family of hydroxylated *s*-triazines metabolites found in plants[24], was clearly negative[25].

Figure 6: SAR Assessment of Mammary Carcinogenic Potential of the Chloro, S-Methyl, Methoxy and Hydroxy-Triazines in Female Sprague-Dawley Rats

					
	**Atrazine** **Positive**	**Simazine** **Positive**	**Propazine** **Positive**	**Terbuthylazine** **Positive**	**Cyanazine** **Positive**
	**Ametryn** **Negative**	NE	**Prometryn** **Negative**	**Terbutryn** **Negative**	NE
	NE	NE	**Prometon** **Negative**	**Terbumeton** **Positive**	NE
	**Hydroxy-Atz** **Negative**	NE	NE	NE	NE

NE = Not Evaluated in chronic bioassays.

3.6 Absorption, Distribution and Elimination of Atrazine

3.6.1 Rodent

Orally administered atrazine is rapidly absorbed and eliminated by the rat, primarily in the urine, independent of dose level and sex. Approximately 73% of the administered dose appeared in urine and 19% in feces. Less than 0.1% was detected in expired air, indicating that the triazine ring remains intact. Excretion data were best fit using first-order elimination kinetics from a two-compartment open system. The elimination half-life from the whole body was determined to be 31.3 hours by the rat. A dominant factor that prolonged elimination was the propensity of atrazine to covalently bind to cysteine sulfhydryls in the rat hemoglobin chain, thus accounting for the second (peripheral) compartment seen in the rat.

The metabolism of atrazine in the rat has been extensively investigated in eight Ciba (now Novartis studies[26]). Atrazine is metabolized in the rat by two primary routes: 1) dealkylation of side chain ethyl and isopropyl groups, which leads to a major urine and fecal metabolite, G-28273, and 2) dechlorination involving conjugation with glutathione to give various mercapturates, mercaptans, sulfides, and disulfides (Figure 7).

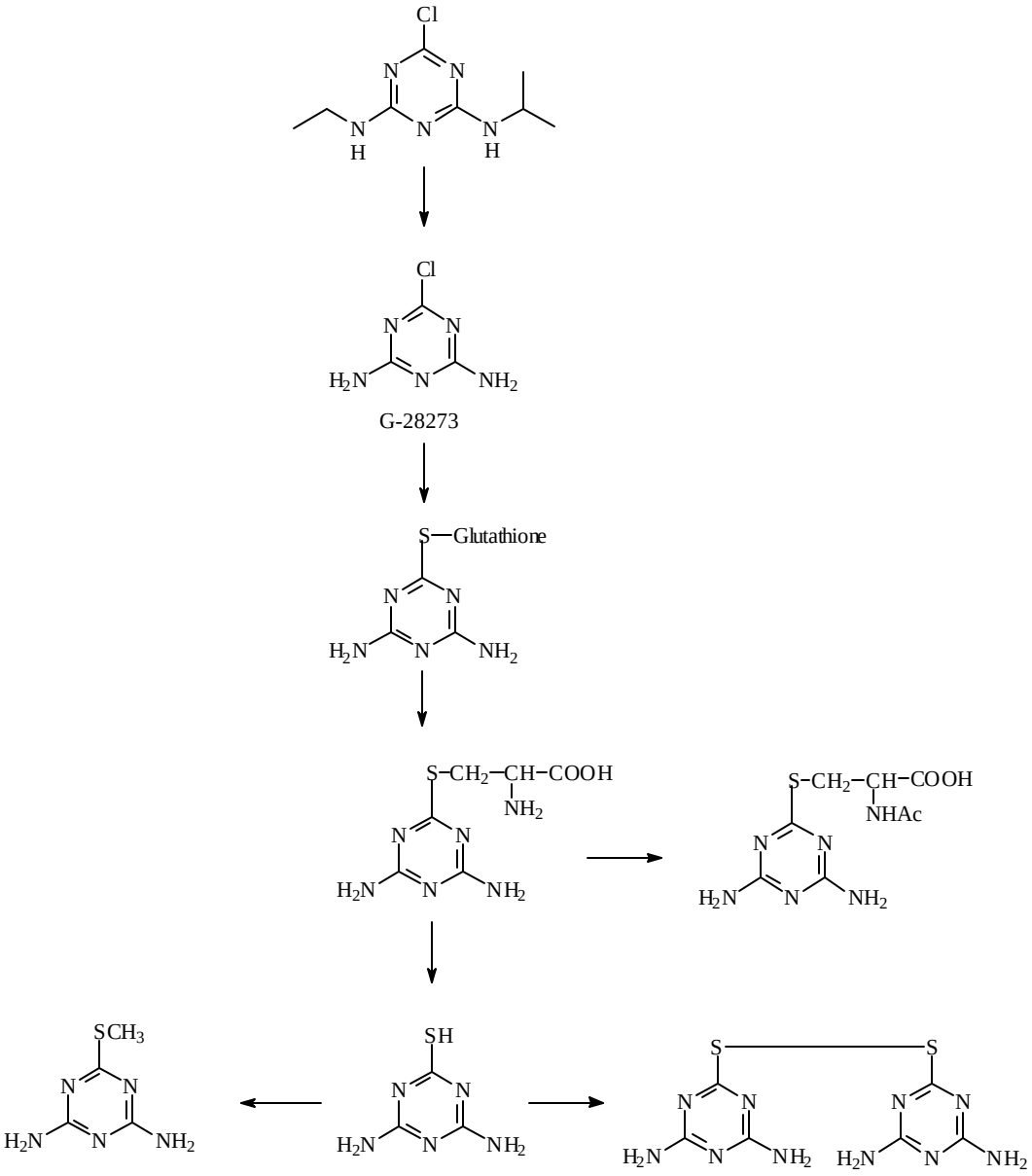
The metabolism of hydroxyatrazine was also studied in the rat, since this compound is a major plant and soil metabolite. Hydroxatrazine was rapidly absorbed and excreted in the urine and feces. The route of excretion was dependent on the dose level; urinary excretion predominated in the low dose and fecal excretion in the high dose. Hydroxyatrazine was transformed primarily (83% of the dose) to other hydroxy dealklyated metabolites.

3.6.2 Human

Atrazine administered orally to six male human volunteers at a dose of 0.1 mg/kg was rapidly absorbed and excreted, primarily in urine.[27a, 27b] Although parent atrazine was not detected in the urine, an average of 14.4% of the administered dose was recovered as chlorotriazine equivalents. The kinetics of urinary elimination of the primary urinary metabolite, G-28273, were best described by single-compartment first order kinetics. The half-life for renal elimination of atrazine in humans was 11.5 hours. The fact that a two-compartment model was not needed to describe elimination kinetics in humans is consistent with the observation that cysteine sulfhydryl groups in human hemoglobin do not bind triazines or their sulfoxides as readily as does rodent hemoglobin[28].

Overall, the results from the rat and human metabolism and elimination studies indicate that atrazine is handled qualitatively similarly in human and non-human primates compared to the rat.

Figure 7: Primary Metabolic Pathway For Atrazine in the Rat



3.7 Summary of the Animal Evidence Factors

The weight of the evidence derived from chronic bioassays was assessed on the basis of a number of factors that may increase or decrease concern with regard to human carcinogenic potential according to the conceptual framework described in the proposed cancer risk assessment guidelines (Table 2).

Table 2: Summary of the Animal Evidence Factors

Carcinogenic Potential (Response Characteristics)	Increased Weight	Decreased Weight
Single Tumor Type vs. Multiple Tumor Types	Mammary and Pituitary (onset only)	Both tumors types are responsive to the same hormonal stimuli
Benign Tumor vs. Malignant Tumor	Malignant tumors (adenocarcinomas)	Benign tumors (fibroadenomas)
Common Tumor vs. Rare Tumor		High spontaneous control incidence; High variability.
Early Onset vs. Elevated Incidence	Early onset	Highly variable onset.
Single Specie vs. Multiple Species		Single specie; Strain-specific
One Sex vs. Both Sexes		One sex
Dose-Response Characteristics Known vs. Unknown		Dose-response well described: NOEL determined
Structure-Activity Relationships	Chloro-s-triazines positive	S-methyl and hydroxytriazines negative; Methoxytriazines:1 negative, 1 positive
Rodent vs. Human Metabolism	Qualitatively similar	Quantitatively different

In summary, the weight of evidence from chronic animal bioassays indicates that:

- Atrazine causes an increased incidence and/or an earlier onset of mammary gland and pituitary tumors in a single sex (female), single strain (SD) and single species (rat);

- The tumors (mammary gland adenocarcinomas and fibroadenomas, and pituitary adenomas) are of the same type as those occurring spontaneously in the SD rat;

- The incidence of the tumors is dose-related and shows a clear dose threshold.

4.0 Potential Direct Modes of Action Underlying the Carcinogenic Response

4.1 Genotoxic Potential

Atrazine has been evaluated for it genotoxicity potential in more than 3 dozen assays including *in vitro* and *in vivo* gene mutation, chromosomal aberration, and other tests. These studies were evaluated [48] and the results are summarized in Table 6.

Table 6. Results of 38 Genotoxicity Assays Conducted on Atrazine.[48]

In Vitro Test Systems		
Gene Mutation	Negative	Positive
Salmonella *typhimurium* (6 studies)	6	
E. *coli* (1 study)		1
Yeast (2 studies)	1	1
HGPRT/V79 Lung Cells (1 study)	1	
Mouse Lymphoma (1 study)	1	
Chromosomal Aberration		
Sister Chromatid Exchange (2 studies)	2	
Chromosomal Aberration (2 studies)	2	
Nucleus Anomaly (2 study)	2	
Other		
Unscheduled DNA Synthesis (2 studies)	2	
In Vivo Test Systems		
Gene Mutation		
Drosophilia Sex-Linked Recessive (3 studies)	2	1
Chromosomal Aberration		
Mouse Micronucleus (1 study)	1	
Mouse Dominant Lethal (4 studies)	3	1
Chromosomal Aberration (8 studies)	7	1
Other		
Mammalian Tests (3 studies)	2	1

The overall weight of evidence from these tests indicates that atrazine is not genotoxic. Six out of 38 mutagenicity studies evaluated were positive. Other studies using the same type of test system were negative. Furthermore, atrazine did not induce a tumorigenic response in ovariectomized female Sprague-Dawley rats[10]. Genotoxic carcinogens like DMBA[68,69] and MNU[70], on the other hand, are capable of inducing mammary tumors in ovariectomized rats.

4.2 Atrazine Metabolites

4.2.1 Chloro-Metabolites

The chlorotriazine metabolites of atrazine (mono and di-dealykylated metabolites) have been profiled toxicologically in standard acute, genotoxicity, developmental studies (rat) and subchronic studies in rat and dog. Overall, the results indicated that these metabolites are toxicologically equivalent to parent. Studies comparing the effects of atrazine on LH and the estrous cycle to the di-dealkylated metabolite of atrazine (DACT) have shown that it is equivalent to parent with respect to the proposed mode of action in both the adult and the developing animal (Laws et al., 2003).[120] These results are consistent with EPA's determination that there is a common mechanism for the chloro-s-triazines including the chloro-metabolites.[121]

4.2.2 Hydroxy-Metabolites

Hydroxyatrazine, which is the major plant metabolite of atrazine has been evaluated in a standard battery of acute, mutagenicity and subchronic toxicity studies. Hydroxyatrazine does not have the same mode of action as does atrazine and is not carcinogenic in the female Sprague-Dawley rat.[122]

4.2.3 N-nitroso-atrazine

N-nitroso-atrazine has not been found in the environment and does not form in under the conditions of pH found in the human stomach.

4.3 Estrogenic Potential

The estrogenic potential of atrazine has been thoroughly examined. The tests for direct estrogenic activity are summarized in Table 7.

Table 7. Evaluation of the Estrogenic Potential of Atrazine Within the Context of the Proposed EDSTAC Screens and Tests[49]

Proposed EDSTAC Screens and Tests		Results
Screen/Test	**Tier I Screen**	
Estrogen Receptor Binding / Transvected Construct		
X[1]	Estrogen Receptor Binding (Uterus)[50, 51]	Negative
	Progesterone Receptor Assay (Uterus)[51,52]	Negative
X	E-Screen (MCF-7 Cells)[51, 53]	Negative
X	Estrogen Receptor Binding (Trans.Yeast Cells)[54, 55]	Negative
Uterotrophic Assay (Estrogen and Anti-Estrogen)		
X	In Vivo Uterotrophic Assay (Estrogen)[51,52]	Negative
X	In Vivo Uterotrophic Assay (Anti-Estrogen)[51,52]	Negative
	Uterine Thymidine Incorporation[52]	Negative
Tier II Tests		
	Endocrine Organ Histology[56]	Negative
	Palpable Masses in Ovariectomized Rats[10]	Negative
	Rat and Rabbit Developmental Toxicity[57, 58]	No indication
X	2-Generation Reproduction Study[59]	No indication

[1] Indicate an EDSTAC Tier 1 Screen or Tier 2 Test

The experimental evidence supports that atrazine has no intrinsic estrogenic potential based on the lack of response in either of the proposed EDSTAC screens[49] (uterine weight, estrogen receptor binding, transvected mammalian and yeast cell models). The Tier II test (2-generation reproduction study) was also negative. Although there was a possible suggestion that atrazine may possess some weak anti-estrogenic activity,[52, 54] this finding was not replicated.[51,55]

4.4 Aromatase

In vitro studies reported by Sanderson have reported that atrazine increased aromatase activity and expression in the adrenocorticocarcinoma cell line H295R but not in the MCF-7 breast cancer cell line, the R2C rat Leydig cell cancer cell line, the carp hepatocyte or JEG-3 placental choriocarcinoma cells (Sanderson, 2000, 2001; 2002,Heneweer, 2004).[123-126] In fact, in the majority of these studies statistically significant induction was only achieved at the highest dose tested. This leads to the conclusion that the inhibition of phosphodiesterase activity may be the result of non-specific high-dose chloro-triazine toxicity. Further, as it has been demonstrated that many human endocrine tissue cancer cells, not only have increased levels of the aromatase gene but they may have a different distribution of aromatase mRNA from normal tissue,[127] it has to be questioned as to whether the aromatase present in the H295R (human adrenocortical carcinoma) cell is representative of normal aromatase.

Atrazine pretreatment of the rats reveals no clear indication that atrazine has the capability of inducing any of the hepatic cytochrome P-450 isoenzymes, including aromatase (CYP19) in the rat [although the CYP19 was not directly evaluated] (Hanioka et al., 1998a).[128] Atrazine may have induced aromatase in a study by Stoker et al. (2000)[129] in male Wistar rats dosed with atrazine for 30 days at 200 mg/kg/day. In a follow-up study to Stoker et al.(2000), Modic et al. (2004)[130] found no effect on aromatase levels in the testis and hypothalamic tissue after treatment with atrazine at doses as high as 200 mg/kg/day at any time point. In addition, Rayner et al. (2004)[131] in perinatal exposure study involving cross-fostering of pups from dams treated with atrazine at 100 mg/kg/day from gestational days 14 through 19, no effect was noted on aromatase messenger RNA levels in the mammary gland at either 33 or 40 days postpartum, except a reduction in aromatase activity was noted from pups potentially exposed to atrazine *in utero* and lactationally. These findings are complimented by evidence of no effect of atrazine exposure on aromatase activity in Zebrafish [up to 112 ppb] (Kazeto et al., 2004)[132], the Xenopus [up to 25 ppb] (Hecker et al., 2003)[133] and alligators [up to14 ppm] (Crain et al., 1997).[134] Therefore, it would appear based on these *in vivo* or *ex vivo* findings that atrazine does not affect aromatase activity in intact animals.

5.0 Indirect Mode of Action of Atrazine in the Aging Female Sprague-Dawley Rat

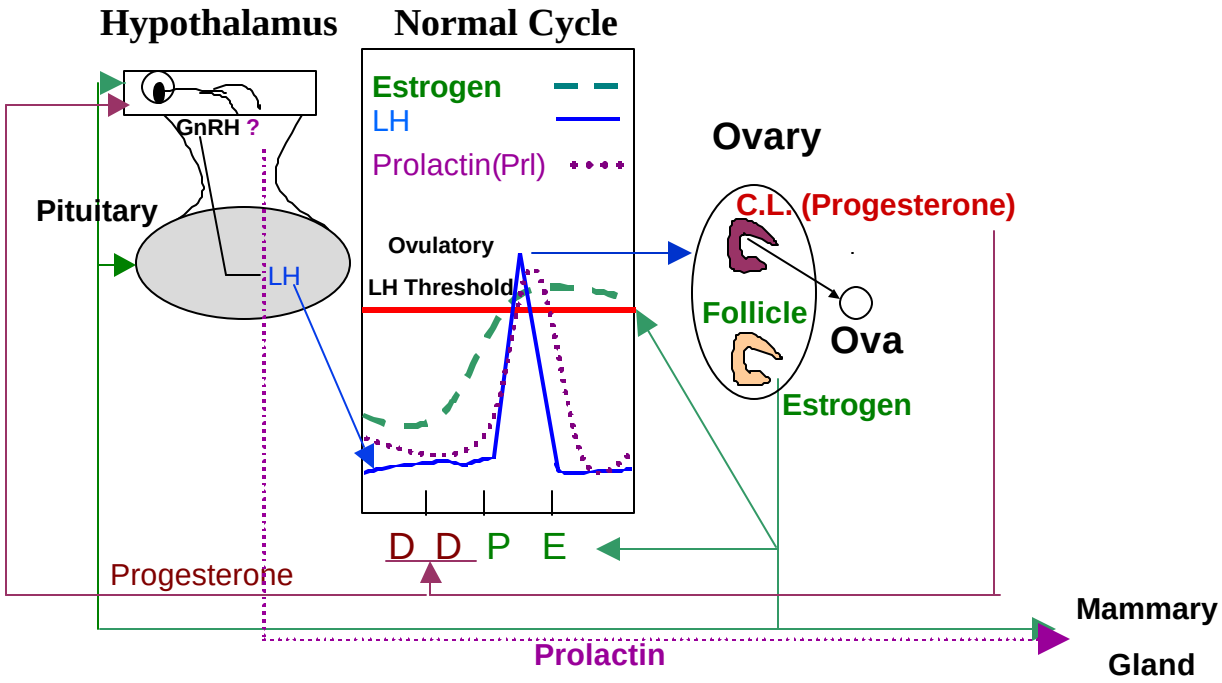
In this section, the research supporting the proposed indirect mode of action leading to the earlier appearance of mammary tumors in female Sprague-Dawley rats administered high doses of atrazine will be presented. The section will end with a determination of whether the proposed mode of action satisfies the elements of proof discussed in the

USEPA Cancer Risk Assessment guidelines (key events identified, strength, consistency and specificity of association, dose-response and temporal consistency, biological plausibility and coherence).

5.1 Conceptual Framework

The conceptual framework that has emerged is that atrazine interacts with molecular targets (membranes, receptors, neurotransmitter systems) within the hypothalamus. This leads to a suppression of the intermittent secretion of gonadotrophin-releasing hormone (GnRH) during critical phases of the normal rodent estrous cycle, which is comprised of one to two days in diestrus (D), one day in proestrus (P) and one day in estrus (E). (Figure 8).

Figure 8: Schematic of the Normal Estrous Cycle and the Ovulatory LH Threshold

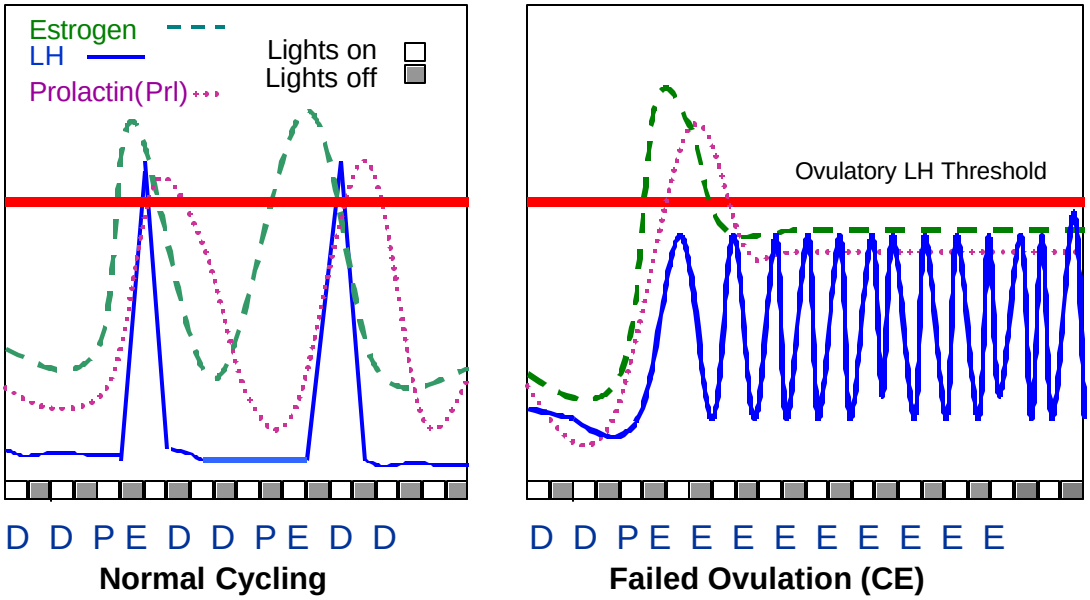


The consequence of this primary event is that luteinizing hormone (LH) released from the pituitary is of insufficient amplitude or duration to trigger the ovulation of developing follicles in the ovary (Figure 9).

The failure to ovulate in the female Sprague-Dawley rat is a key event that leads to prolonged exposure to endogenous estrogen and/or prolactin for each additional day that the animal spends in a state of estrus or diestrus. Ovulation in the Sprague-Dawley rat has a biological threshold. Either the animal ovulates or it does not. Either the atrazine dose is sufficient to suppress LH to block ovulation or it is not.

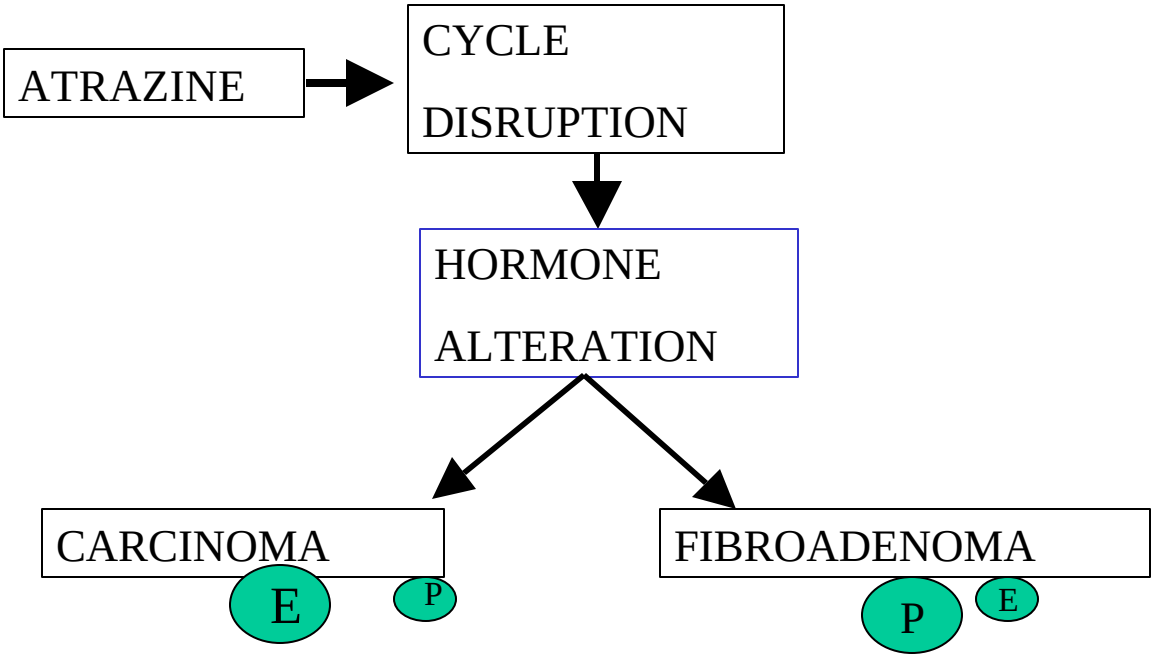
The net consequence of ovulatory failure is that the mammary gland is hyperstimulated by estrogen arising from the follicle and prolactin produced by the pituitary. Over a sufficiently long time period, this hyperstimulation translates into a proliferative response in the mammary gland characterized by the development of adenocarinoma (high estrogen, moderate prolactin levels) or fibroadenoma (high prolactin with a background of estrogen) as illustrated schematically in Figure 10.

Figure 9: Schematic of Normal and Constant Estrus in Sprague-Dawley Rats



The cascade of events triggered by high doses of atrazine administered to female Sprague-Dawley rats is qualitatively similar to that observed in spontaneously aging Sprague-Dawley female rats. The major difference is that in atrazine-treated female Sprague Dawley rats, the pituitary/hypothalamic failure occurs slightly earlier. The Fischer-344 rat is refractory to these changes because the reproductive aging process is dissimilar from that occurring in the Sprague-Dawley rat.

Figure 10: Cascade of Key Events Leading to Mammary Tumors in SD Rats.

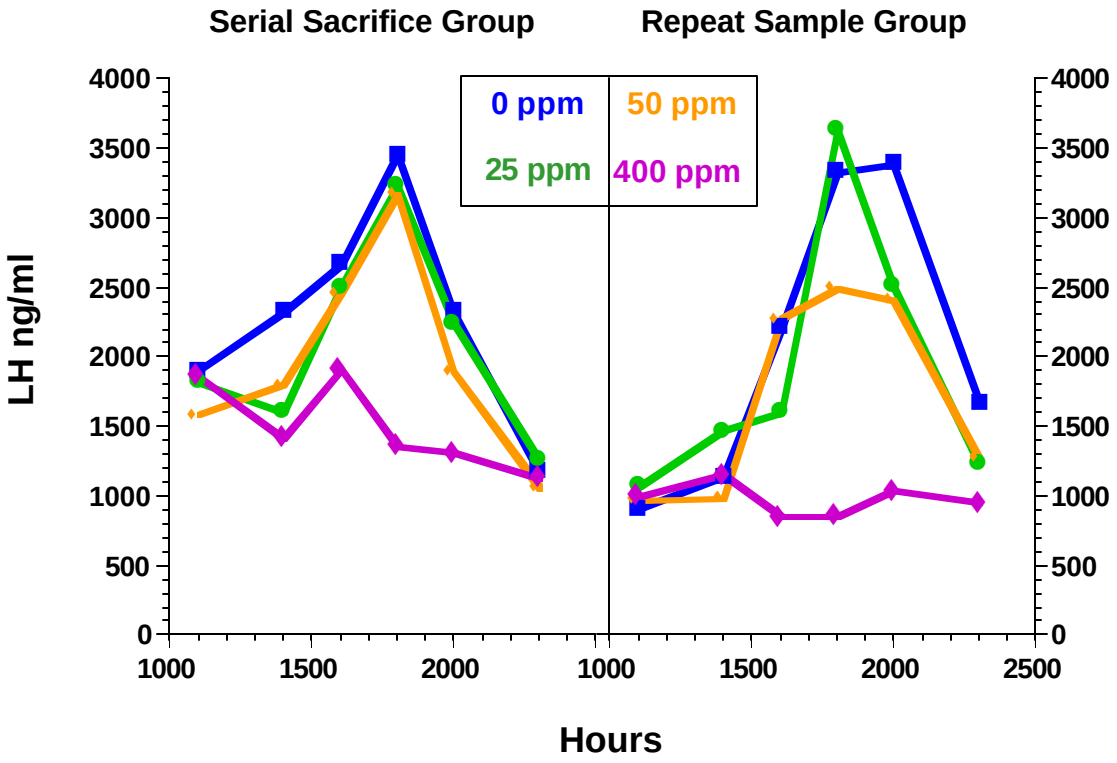


5.2 Effect of Atrazine on LH and Gonadotrophin Releasing Hormone (GnRH)

Studies conducted by Cooper et al.[60,61] indicated that 2 to 3 week pretreatment of Long-Evans rats with high doses of atrazine (75 to 300 mg/kg/day) suppressed both serum LH and prolactin surges in ovariectomized animals; (Long-Evans and the Sprague-Dawley rats are outbred strains derived from the Wistar rat). Daily intraperitoneal administration of atrazine to ovariectomized rats at a dose level of 200 mg/kg (but not 50 mg/kg/day) for 3 days markedly inhibited episodic pulses of LH and significantly suppressed the serum LH level relative to controls[62]. These results have been confirmed in short duration studies.[63,64]

A six month feeding study conducted by Corning Hazleton, Inc.[65] indicated that LH levels were significantly suppressed only at feeding levels (400 ppm) where a mammary tumor response was observed in chronic bioassays[3] (Figure 11). No significant effect of atrazine treatment on the LH surge was observed at feeding levels ($\leq$ 50 ppm). This feeding level was not associated with an earlier appearance and/or an increased incidence of mammary tumors in a recent chronic bioassay on atrazine.[10]

Figure 11: Effects of Atrazine on the LH Surge in Female Sprague-Dawley Rats

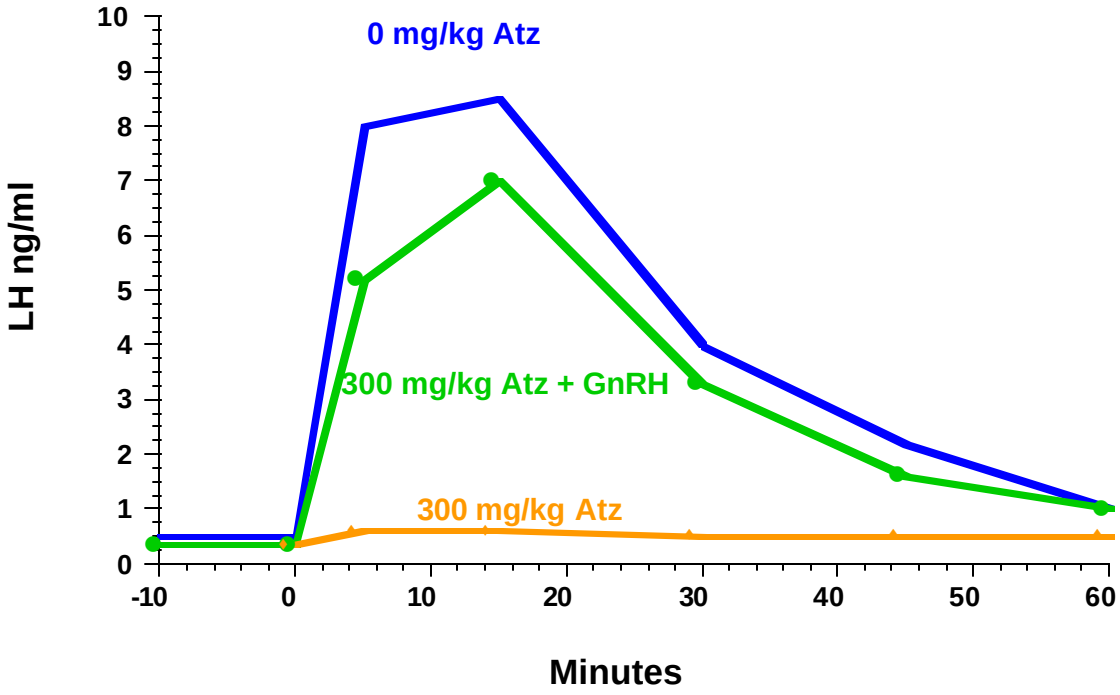


The ability of atrazine to inhibit the ovulatory surge of LH indicates a site of action at either the level of the hypothalamus or the pituitary. Action within the hypothalamus could deprive the pituitary of the GnRH signal necessary for LH release, whereas effects within the pituitary might diminish the secretory response to that signal or processes involved in the biosynthesis of LH. Normal concentrations of LH and prolactin were found in the pituitary, suggesting that the hormones had not received the appropriate release signals.[61] High doses of atrazine led to a reduction in hypothalamic norepinephrine[60] suggesting a hypothalamic site of action.

The retention of pituitary responsiveness to GnRH stimulation after atrazine treatment (300 mg/kg/day x 3 days) was confirmed by the demonstration that a normal serum LH surge was reinstated in atrazine-treated animals if GnRH (50 ng/kg) was administered[73] (Figure 12).

A recent study completed in a group of ovariectomized, estrogen-replaces Rhesus monkey administered atrazine at a daily dose of 25 mg/kg/day, a dose that approached or exceeded the maximum tolerated dose in the monkey, did not provide convincing evidence of an effect of atrazine on the LH surge (Covance, 2004).[135]

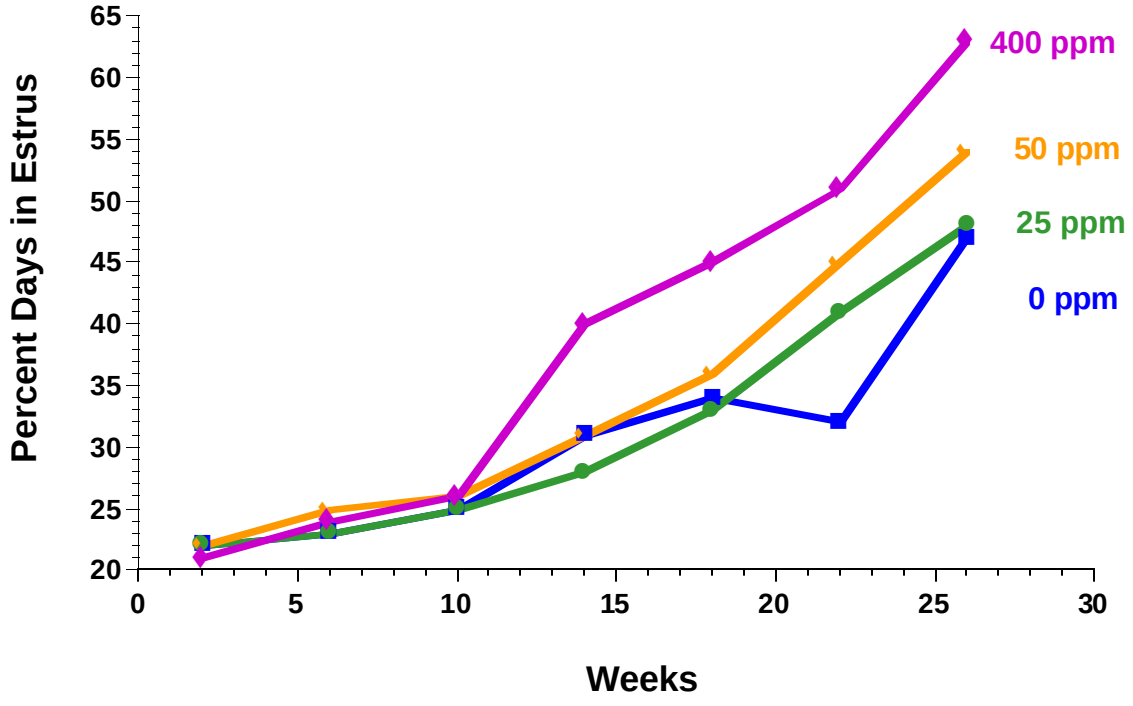
Figure 12: Effects of GnRH Replacement on the Atrazine-Induced LH Surge Suppression



5.3 Effect of Atrazine on the Estrous Cycle

Animals in the 6-month study[65] conducted to evaluate the effect of atrazine treatment on the LH surge had daily vaginal smears collected in alternating 2-week blocks of time. Based on cytological characteristics, the smears were classified for each day as being either proestrus (P), estrus (E) or diestrus (D). The proportion of days spent in each stage was calculated and the results are summarized in Figure 13 and presented for individual animals in the control and high dose groups in Figures 14 and 15, respectively.

Figure 13: Summary of the Estrous Cycle Data in Atrazine Treated SD Rats



The results indicate that high dose atrazine animals begin to develop abnormal estrous cycles after approximately 10 weeks of treatment as indicated by an increased proportion of time spent in estrus. Control animals also displayed an increased number of abnormal days in estrus after 10 weeks but not to the same extent as did the high-dose atrazine animals. There was no effect of treatment on the estrous cycle at feeding levels of $\leq$ 50 ppm.

Figure 14: Daily Estrous Cycle Data from Control SD Rats

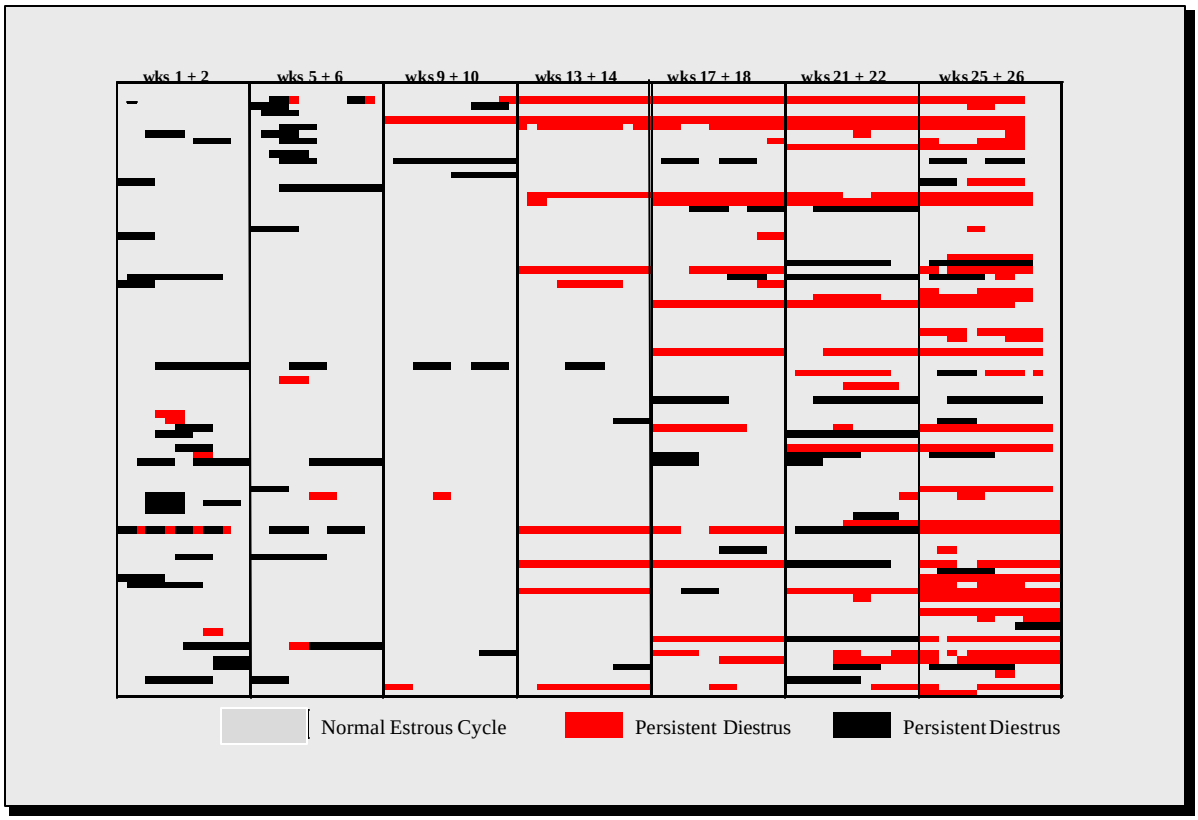
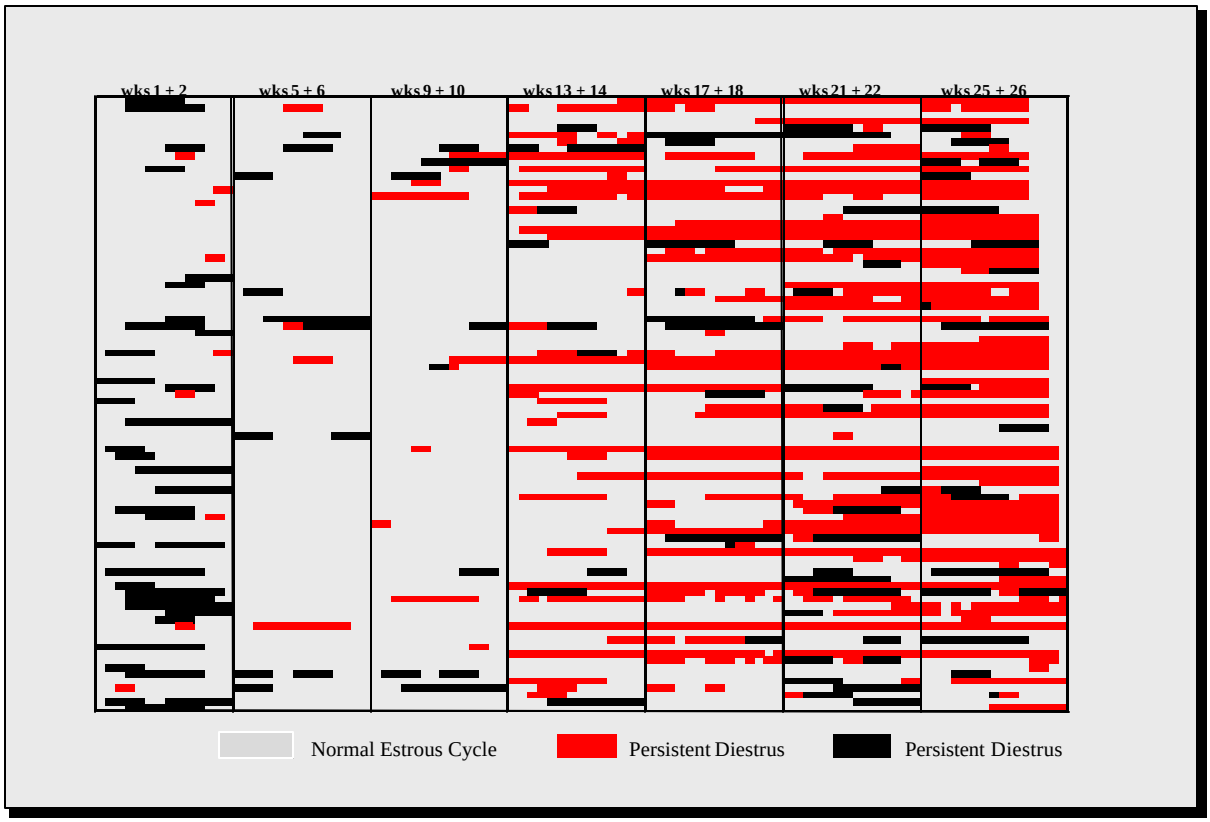


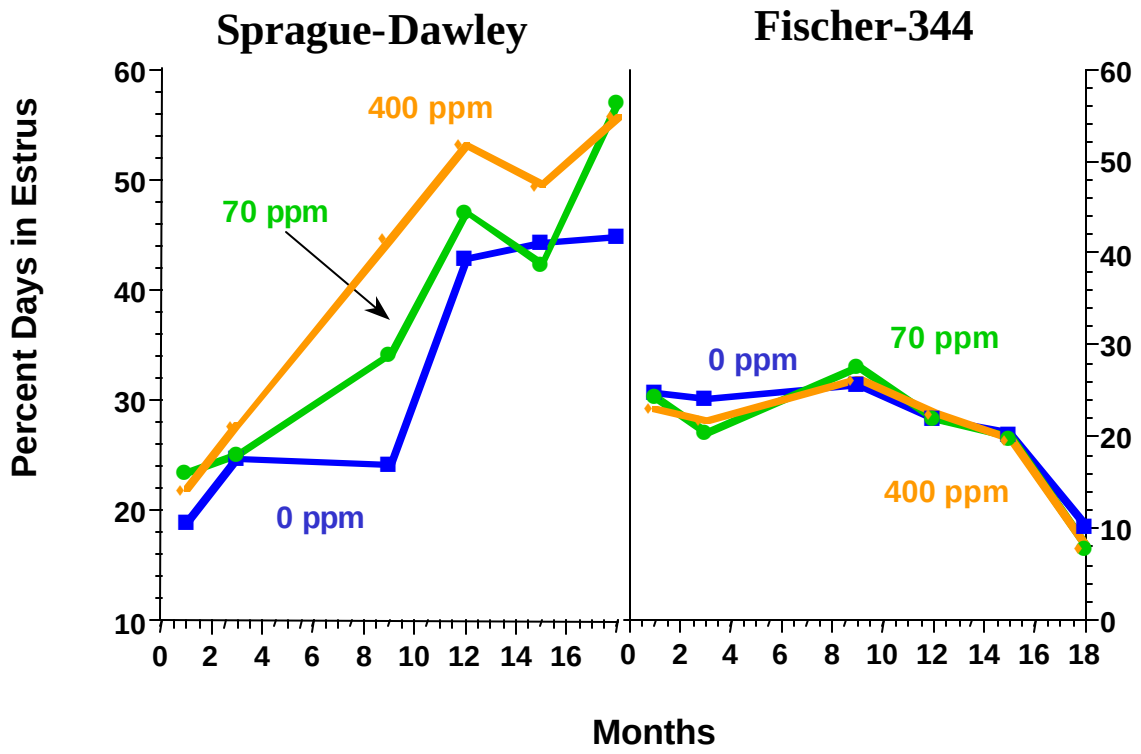
Figure 15: Daily Estrous Cycle Data from 400 ppm Atrazine



Atrazine Treated Rats

The effects of atrazine on estrous cycle and tumor response in a chronic bioassay was investigated in a chronic study in the female Sprague-Dawley[4] and Fischer-344 rats.[6] High dose female Sprague-Dawley rats fed 400 ppm atrazine (~30 mg/kg/day) displayed an earlier appearance (after 9 months of treatment) of altered estrous cycles characterized by an increase in the percent of days in estrus. In contrast, female Fischer-344 rats fed up to 400 ppm of atrazine exhibited no effect on the percent days in estrus (Figure 16).

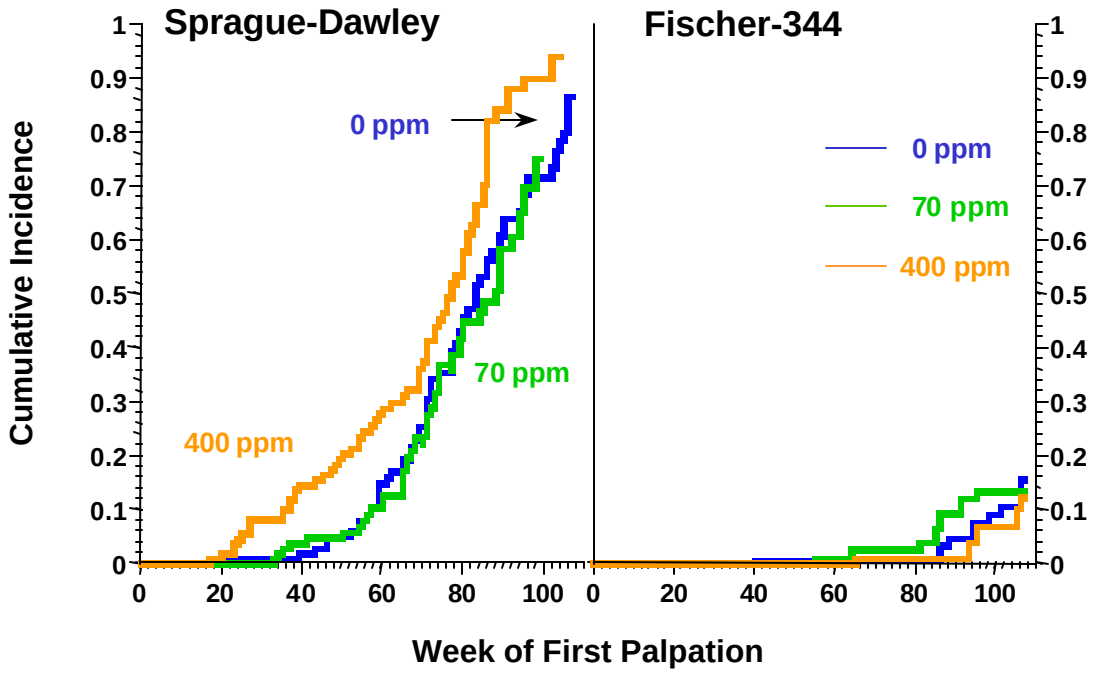
Figure 16: Effect of Atrazine on the Estrous Cycle of SD and Fischer-344 Rats



The mammary tumor data from these two studies is shown in Figure 17. The results indicate that female Sprague-Dawley rats display an earlier disruption of the estrous cycle than do control animals and develop mammary tumors earlier. Disrupted estrous cycles precede the appearance of mammary tumor development in both the control and treated groups; the effect of high doses of atrazine is to make both of these events occur earlier.

The control Fischer-344 female rat does not display abnormal estrous cycles until late in life and it does not develop a high incidence of mammary tumors. High doses of atrazine have no effect on either the estrous cycle or mammary tumor incidence.

Figure 17: Kaplan-Meier Plots of the Cumulative Mammary Tumor Incidence in Female Sprague-Dawley and Fischer-344 Rats Treated with Atrazine[3,4,5,6]



6.0 Dose Response Modeling of Mammary Tumor Development in SD Rats

6.1 Biologic Basis for Model Development

Statistical association between dose and response are best considered by evaluating the dose-response relationship for all groups of animals over the entire study period. Using shorter intervals of time may weaken the evaluation of the relationship between dose and response. This would especially be true if the time-to-tumor is dependent on factors that are operative over the entire study period such as is the case for endocrine-mediated mammary tumor development in the female Sprague-Dawley rat.

Selecting a surrogate for dose that accounts for more of the observed data than dose alone indicates that the predictor variables (See Section 7.2) are more directly related to the response than is the dose. This situation may occur for multistage processes where dose expressed in terms of mg/kg of the compound is only directly linked to key events that occurs early in the process. For atrazine, LH surge suppression is considered such a key event.[62, 65, 71] The sequels to LH surge suppression in the female Sprague-Dawley rats include estrous cycle disruption, and the occurrence of persistent diestrus and estrus.[72] Secondary effects include hyperstimulation of the mammary gland and pituitary with endogenous estrogen (persistent estrus) and/or prolactin (pseudopregnancy).[73] These events lead in turn to mammary gland pathology in the form of carcinoma development (estrogen-mediated)[74] or abnormal lobular-alveolar development, ductal dilation, acinar

formation and increased secretory activity, galactocele formation and fibroadenoma development (prolactin mediated).[56,75]

Estrogenic stimulation of the pituitary also leads to pituitary hyperplasia and the development of benign tumors.[76] Under these conditions, the pituitary secretes high levels of prolactin from lactomorphs, which are no longer under the inhibitory control of the dopaminergic systems located in the hypothalamus.[77,78] Elevated prolactin levels derived from this source further stimulate mammary lobular development, which may ultimately be expressed as an earlier appearance and/or increased incidence of fibroadenomas.[79,80]

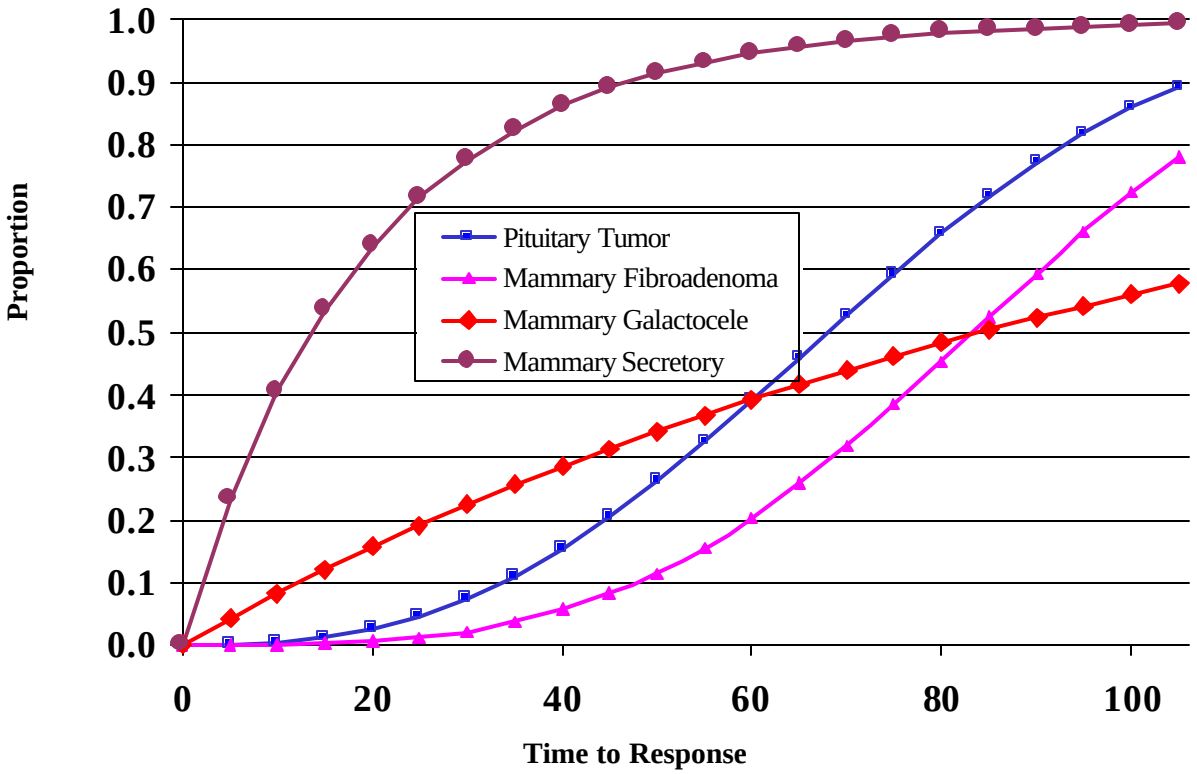
6.2 Multistage-Weibull Time-to-Tumor Model

An assessment of estrous cycle data through 50 weeks was conducted.[66] as well as other indicators of hormonal imbalance in animals from a recent Covance study.[10] Thirty-five predictor variables including the percent of time spent in estrus or diestrus, the percent of the time spent in abnormal estrus or diestrus and whether the animals had pituitary tumors, mammary secretory activity or galactoceles were evaluated and compared to background and the ppm atrazine dose using a likelihood ratio. The probability that an animal would develop a fibroadenoma or an adenocarcinoma by time *t* was evaluated using a multistage Weibull time-to-tumor model. The likelihood of observing the study results if the fitted model were true was calculated for each scenario and compared to the likelihood based on background (i.e. no dose metric was employed) or the ppm atrazine dose. The magnitude and the statistical significance of the difference between these values were calculated.

6.2.1 Fibroadenoma

The results indicate that the fitted model for fibroadenomas is significantly more likely to reflect the observed data (3 to 6 fold increase) when days in diestrus, days in estrus or percent abnormal days in estrus are used as the dose metric in place of ppm atrazine. More significantly, however, is the observation that mammary secretory activity increases likelihood by a factor of greater than 1×10^{16}. Restated in another way, the presence of secretory activity in the mammary gland is much more predictive of whether an animal developed fibroadenomas in this study than knowing what dose of atrazine was administered. Adding information on whether the animal developed a galactocele, increased the likelihood by a factor of 7000. Adding information on whether the animals had a pituitary adenoma resulted in an 189-fold increase in the likelihood while the addition of information on the proportion of abnormal days in diestrus during weeks 1-26 resulted in a modest 10-fold increase in the likelihood. No further additions resulted in statistically significant increases in the likelihood. The relationship between predictor variables and the time to fibroadenoma development is illustrated in Figure 18.

Figure 18: Prediction of the time to fibroadenoma development compared to the time to develop mammary secretory activity, galactoceles, and pituitary tumors



These results are in agreement with research literature indicating that endogenous prolactin in the presence of a background estrogen play an important role in female Sprague-Dawley fibroadenoma development.

6.2.2 Adenocarcinoma

The fitted model for adenocarcinomas was significantly more likely to reflect the observed data (153-fold increase) when the percent or total number of abnormal days in estrus during weeks 1-26 are used as the dose metric in place of ppm atrazine. More modest increases in the likelihood were observed when days in estrus were evaluated for other time periods. Having information on the days spent in diestrus, or whether the animals had mammary secretory activity, galactoceles or pituitary adenomas did not increase the likelihood of the model prediction over just knowing the ppm atrazine dose. Restated in another way, knowing the percent of time spent in estrus or in abnormal estrus was significantly more predictive of whether an animal developed adenocarcinoma in this study than knowing the dose of atrazine administered.

6.2.3 Differential Prediction of Mammary Adenocarcinomas and Fibroadenomas

Predicting when an animal was likely to develop a mammary fibroadenoma was significantly increased if information on mammary secretory activity, the presence of galactocele or pituitary tumors, the percent of time spent in diestrus or abnormal diestrus and the percent time spent in estrus was known. None of these variables were important for prediction mammary adenocarcinomas except for data on the percent time spent in estrus or abnormal estrus. These results, which are derived from mathematical modeling, are consistent with expectations based on an understanding of factors that impact adenocarcinoma and fibroadenoma development in the female Sprague-Dawley rat.

7.0 Relevance of the Proposed Mode of Action to Man

The relevance of the mode of action underlying mammary tumor development in the female Sprague-Dawley rat to humans will be considered. Similarities and differences in cycle control mechanisms, reproductive aging processes and the response of humans to drugs that cause elevated prolactin levels (reserpine) or suppress LH (birth control pills) are discussed in detail in the attached report[67] and are summarized as follows.

7.1 Comparison of the Reproductive Cycles

In the rodent, the estrous cycle is short and the pre-ovulatory LH surge is brief, timed by the light cycle and depended on the brain. The brain plays a deterministic role in the LH surge in rodents. Every afternoon during a critical period, a brain signal for LH secretion occurs that is driven by the increased activity of norepinephrine neurons.[82-85] As such, selective blockage of this increased activity of norepinephrine neurons during this brief period blocks the pre-ovulatory LH surge.[86]

The human menstrual cycle is long, exhibits a protracted preovulatory LH surge and ends with menses due to the death of the corpus luteum and the resulting decline in estrogens and progestins. The driving force for the pre-ovulatory LH surge in women is ovarian estrogen secretion. [87] The role of brain regulation of GnRH in the pre-ovulatory LH surge is permissive in women and other primates.[87-90] Indeed, the entire menstrual cycle can be recapitulated in rhesus monkeys in whom the source of GnRH has been destroyed, by exogenous administration of pulses of the GnRH. [87-90] In contrast to the observations in rodents, inhibitors of norepinephrine neurotransmission do not affect the pre-ovulatory LH surge in women or other primates. [91,92]

7.2 Comparison of Reproductive Aging

Reproductive aging in rodents and women also is distinctively different. In female SD rats, reproductive senescence is a result of a breakdown of the brain regulation of the LH surge, while the ovaries are functional very late into life. [93,94] The decline in reproductive function is primarily a result of the inability of brain norepinephrine neurons to transmit the estrogen signal to GnRH neurons.[83-85,94] The inability to stimulate a pre-ovulatory LH surge results in the maintenance of ovarian follicles and the persistent secretion of

estrogens. Sequentially, the increased secretion of estrogens causes a persistent state of hyperprolactinemia.[78] Thus in the SD rat, reproductive senescence is characterized by persistent hyperestrogenemia and hyperprolactinemia with low levels of LH and follicle stimulating hormone (FSH).

In women, reproductive aging is characterized by exhaustion of ovarian follicles and the resulting menopause.[95] During the menopause, the ability to induce a pre-ovulatory LH surge is normal, but estrogens, the driving force for the cycle, are absent. Post-menopausal estrogens and prolactin are very low, but LH and FSH secretion in women remain high. The major differences between the human and non-human primate menstrual cycles and the Sprague-Dawley estrous cycle are summarized in Table 8.

Table 8. Comparison of the Menstrual Cycle in Women and the Estrous Cycles in SD Rats

Menstrual Cycle in Women	**Estrous Cycles in SD Rats**
Estrogens are elevated for 20 of 28 days of the cycle	Estrogens are elevated for only 1.5of 4 days of the cycle
LH surge lasts for 2.5 to 3 days	LH surge lasts for 4 to 8 hours
LH surge is timed by ovarian follicles	LH surge is timed by the brain
LH surge has no critical period for activation	LH surge have a well define critical period
Norepinephrine antagonists do not block the pre-ovulatory LH surge	Norepinephrine antagonists effectively block the pre-ovulatory LH surge
Brain regulation of LH surge is preserved, but ovarian follicle depletion causes menopause	Aging of the brain regulation of LH surge is critical to reproductive senescence
Post-menopausal decline in prolactin, and increase in LH and FSH	Senescent-related increase in prolactin and decrease in LH and FSH
Post-menopausal decline in estrogens	Senescent-related increase in estrogens
Low spontaneous incidence of mammary tumors	High spontaneous incidence of mammary tumors
Central Inhibition of GnRH pulses during development	No developmental inhibition of GnRH pulses
Estrogen inducible estrogen responsive element (ERE) in the human GnRH gene	No ERE in the rodent GnRH Gene

7.3 Comparison of the Effects of Atrazine Treatment in Female SD Rats with Polycyctic Ovarian Syndrome in Women

No single etiology is known for polycystic ovarian syndrome (PCO) of women.[96,97] The syndrome, usually occurring in the 3rd decade of life, is characterized by ovulatory failure and a substantial ovarian enlargement containing numerous unovulated follicles. There is an increased level of serum androgens [98,.99] and ovarian androgen secretion.[100] Marked hirsutism is a common symptom. [101] Abnormal enzymatic control of steroidogenesis, in the ovary or the adrenal cortex, is a suspected cause of the hyperandrogenism. [101] In addition, an increase of pituitary LH, but not FSH, secretion is frequently noted,[96,98,99,102,103] that results from an increased frequency and amplitude of LH pulses [99,103-105] and increased pituitary sensitivity to GnRH. [102,106]

A summary of comparison is presented in Table 9. PCO is basically an environment of high LH and high androgens, while the ATR-treated rat has diminished LH, persistent estrogen secretion and low androgens. In PCO, LH pulse amplitude and pituitary sensitivity to GnRH are increased; in ATR-treated rats, LH pulse amplitude is inhibited and pituitary sensitivity to GnRH is unchanged. Obesity and diabetes are correlates of PCO patients, but ATR-treated rats lose weight. PCO is associated with later development of endometrial cancer but not breast cancer, while ATR-treated female SD rats have an increased incidence of mammary tumors later in life, but no cancer of the reproductive tract.

Table 9. Comparison Of Polycystic Ovarian Syndrome In Women With Chronic High Dose Atrazine Treatment In Female SD Rats

PCO Syndrome	Atrazine Treatment
Elevated LH Secretion	Reduced LH Secretion
Increased LH Pulse Amplitude	Decreased LH Pulse Amplitude
Increased Pituitary Sensitivity to GnRH	No Change in Pituitary Sensitivity to GnRH
Commonly Correlated with Obesity	Correlated with Weight Loss
Increased Androgen Secretion	Androgens Levels Decreased or Unchanged
Association with Endometrial Cancer	Not Associated with Endometrial Cancer
Not Associated with Breast Cancer	Associated with Mammary Tumors
Does not Resemble Menopause	Resembles Reproductive Senescence
Non-neuroendocrine Etiology	Neuroendocrine Etiology

7.4 Comparison of the Effects of Atrazine Treatment in Female SD Rats with Hypothalamic Amenorrhea in Women

Under a number of circumstances, the human female menstrual cycle fails because of inadequate hypothalamic-pituitary support for ovarian function. Because of the likely central site of origin of these syndromes, they are grouped together here under a single designation of "hypothalamic amenorrhea".

CNS-associated amenorrhea can arise from excessive physical or psychological stress,[107-110] excessive exercise or training, [109,111] starvation and/or severe weight loss and anorexia nervosa.[109]

Pituitary gonadotropin secretion is pulsatile because hypothalamic GnRH release is also pulsatile.[112,113] Nearly every type of hypothalamic amenorrhea is due to reduced amplitude and/or frequency of GnRH pulses.[108,112,114,115]

The significant difference between the human and rat conditions is the respective responses to CNS disruption of gonadotropin secretion. Human amenorrhea has extremely low estrogen levels, while the atrazine-treated SD rat maintains moderate-to-elevated estrogen levels. This difference occurs because, when the rat LH surge is blocked or blunted, the hypothalamic centers repeat their attempt to produce a suprathreshold LH surge on the following day. This pattern may continue for many weeks or months.[116]

In the human, a blocked or blunted surge, seen for example, with oral contraceptive use, is not followed on successive days by additional surges. As a result, hypothalamic amenorrhea in women is associated with reproductive atrophy, a severe decline in estrogen secretion and is not associated with mammary tumors. By contrast, in the female SD rat, atrazine treatment is associated with reproductive senescence characterized by increase exposure to estrogens and prolactin and a resulting increase in mammary tumors.

7.5 Sensitivity During Development

In addition to evaluating the effect of atrazine on the LH surge in adults, several studies have been conducted to evaluate the effect of atrazine during develop. These studies are summarized in Figures 19, which is taken from Table 3-2 (p. 72) of EPA's Preliminary Draft Hazard and Dose-Response Assessment-atrazine (USEPA, 2000) where the EPA summarized the relationship between dose and duration of treatment for various endocrine-related parameters in female Sprague-Dawley rats exposed to atrazine. These data indicate that

1) Changes in LH provide the most sensitive indicator of the effect of atrazine on the endocrine system in the female SD rat and
2) When female Sprague-Dawley rats are exposed to atrazine at a younger age and/or for a shorter duration of time, the no observed adverse effect level (NOAEL) increases.

Figure 19-Summary of NOELs/LOELS from Studies of Different Durations

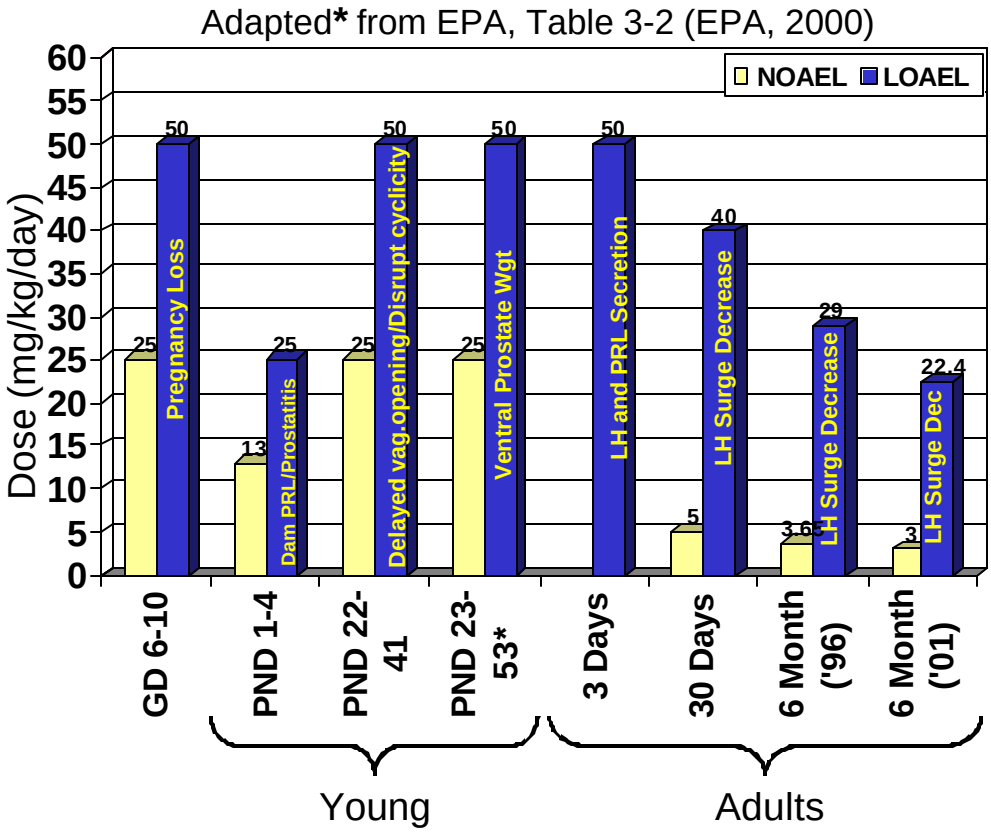
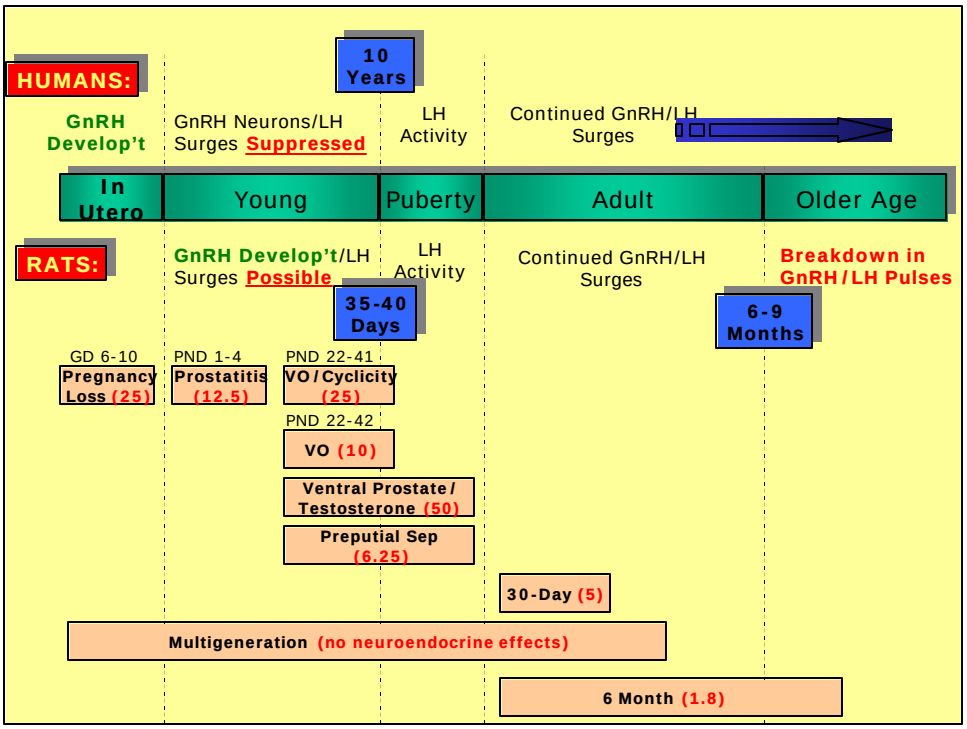


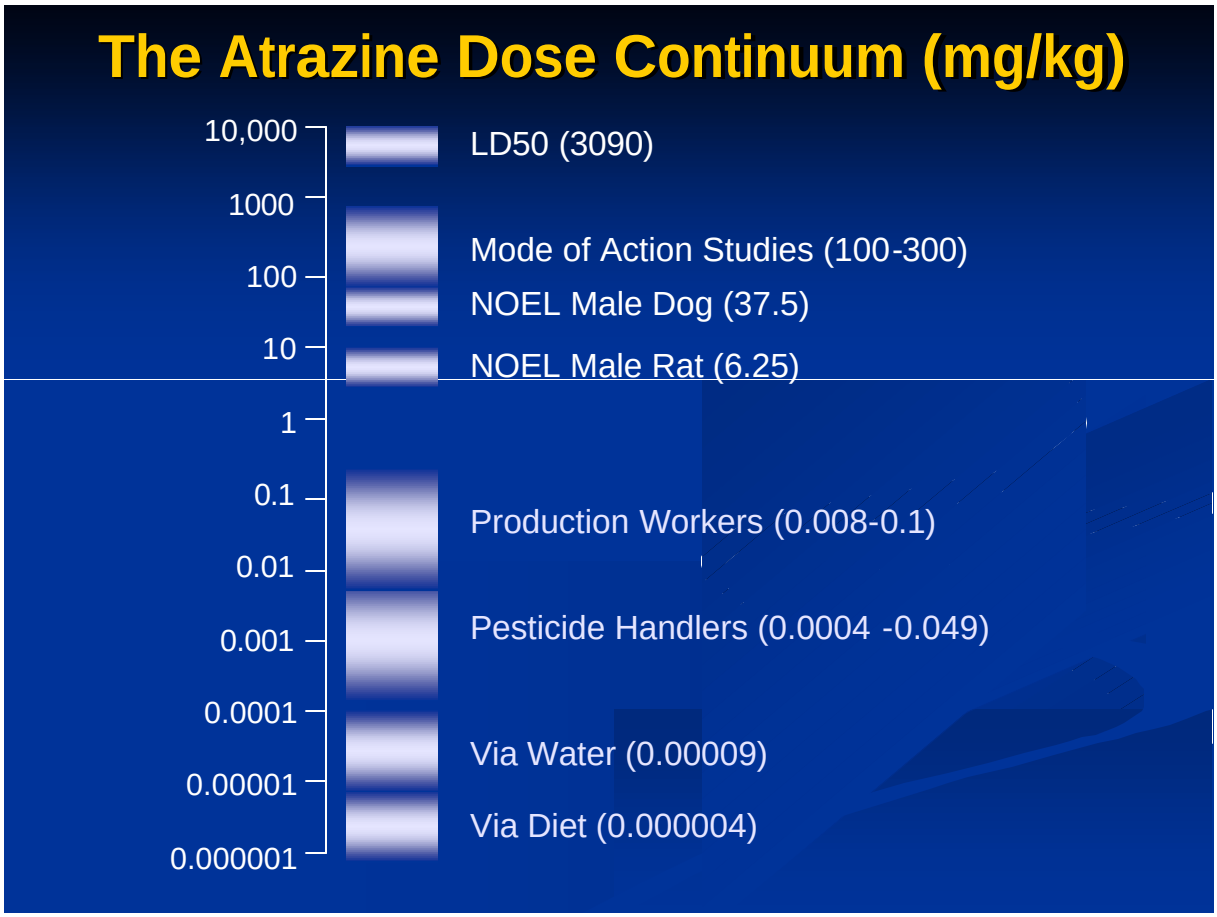
Figure 20 – Schematic of Development in Rodents and Humans



Thus, during development, the Sprague-Dawley rat is actually less sensitive to the endocrinological effects of atrazine than is the adult. Furthermore, there is a period of time from post-lactation until puberty when the pituitary hypothalamic mechanisms that controls the onset of puberty and ovulation in the adult, is quiescent and not sensitive to atrazine. This is true for both rodents and primates (Veldhuis, 1996 Terasawa et al., 2001).[136-137]

8.0 Magnitude of Exposure Relative to Toxicological NOELS

The figure provided below presents the estimated dose continuum for exposure to atrazine in manufacturing and agricultural workers in drinking water (overestimated at 3 ppb) and from all dietary sources. The data indicate that exposure to atrazine for the general public is 4 to 6 orders of magnitude lower than the NOELS from the most sensitive species in toxicity studies and 2 to 4 orders of magnitude lower than exposure for manufacturing and agricultural workers. At these levels or exposure, the EPA has determined in the Interim Re-registration Eligibility decision for atrazine that there would be no toxicological effects of atrazine.



9.0 Carcinogenic Classification of Atrazine Based on the Weight of the Evidence

This document has summarized the results from analyses of the available information relevant to the carcinogenic potential assessment of atrazine. Factors necessary to address the carcinogenic classification in accordance with the EPA's Proposed Guidelines for Carcinogen Risk Assessment (July 1999) include the following elements:

- An assessment of animal and human evidence;
- An evaluation of alternative modes of action;
- The identification of key events underlying the proposed mode of action;
- An evaluation of the biological plausibility of the mode of action;
- An evaluation of the temporal coherence of the key events outlined in the mode of action;
- A dose-response assessment and the identification of key event in the female SD rat;
- Identification of the biological basis of the threshold for key events observed in the SD rat;
- Relevance of the mode of action for humans at doses which cause tumors in SD rats;
- Relevance of the mode of action for humans at environmental exposure levels; these levels are several orders of magnitude below no observed effect level in the female SD rat.

Based on the total weight of the evidence, the appropriate classification for atrazine is "Not Likely to be Carcinogenic to Humans". This classification is consistent with the classification applied by other major international science and regulatory agencies around the world. [117, 118].

Furthermore, an independent expert panel has recommended that human exposure to atrazine should be regulated using a Margin of Exposure approach based on NOEL'S or benchmark doses derived from chronic bioassay endpoints which have been identified as key events in the mode of action (e.g. LH suppression).

The rationale underlying these conclusions are presented in Tables 10 and 11.

Table 10: Summary of the Totality of the Evidence

Animal Evidence	Increased Weight	Decreased Weight

Mammary Tumors	• Early appearance or increased incidence of mammary tumors in female SD rats administered high doses of the chloro-s-triazines.	• Highly variable, spontaneous incidence in female SD rats.
		• Response restricted to 1 species, 1 strain, and 1 sex.
		• SAR generally negative for hydroxy, s-methyl, methoxytriazine.
		• Biological threshold established.
		• Direct modes of action discounted.
		  • Genotoxicity
		  • Estrogenicity
		• Indirect mode of action established
		  • Key events identified
		  • Biologically plausible
		  • Consistent dose-response
		  • Temporally consistency
		  • Consistent with literature

Human Evidence	**Increased Weight**	**Decreased Weight**
Epidemiologic Studies **Human Relevancy**		• Long history of safe use • Many published studies (peer review) • No causal association established. • GnRH control of ovulation different • Reproductive aging different • Prolactin has no role in breast cancer • LH suppression in women on birth control pills does not lead to a high estrogenic state. • Polycystic Ovarian Syndrome not a suitable model (mechanism unknown, LH elevated, estrogen decreased). • Hypothalamic Amenorrhea not a suitable model (mechanism unknown, estrogen levels decreased)

Table 11: EPA Cancer Classification Criteria

EPA Category	Main Criteria	Weight of the Evidence for Atrazine
Carcinogenic In Human	**Causal Association between human cancer and exposure established by conclusive epidemiological data.**	**No Causal Association.**
Likely to be Carcinogenic in Humans	**Evidence of association between human cancer and exposure.** **Strong Experimental Evidence Of Carcinogenic Potential In Animals** **Mode of Action Relevant to Human**	**No Evidence of Association** **Weak Experimental Evidence of Carcinogenic Potential in Animals** **Mode of Action not Relevant to Humans**
Suggestive Evidence of Carcinogenic Potential in Humans	**Suggestive but Insufficient Animal Evidence:** • **Marginal Tumor Increase** • **High Spontaneous Background** • **One Species, One Sex** • **More Data Required**	**Comprehensive/Sufficient Animal Data Mammary Tumor Response Limited to:** • **One Species** • **One Strain of Rat** • **One Sex** • **Response Prevented by Ovariectomy** • **Mode of Action Known** • **Biological Threshold Established**
Data are Inadequate for Assessing the Human Carcinogenic Potential	• **Inadequate Data** • **Conflicting Data**	**Comprehensive Data Sets Available** • **Human Epidemiology Studies** • **Human Exposure Data** • **Animal Bioassay Data** • **Animal Mode of Action Data**
Not Likely to be Carcinogenic to Humans	**Lack of Effect Demonstrated in Human** **Animal Studies Negative** **Mode of Action Not Relevant** **Carcinogenic Response Not Likely Below a Defined Doses**	• **40 Years of Manufacture & Use:** • **Follow-Up Epidemiology Study:34 Years** • **Only positive in the female SD Rat;** • **Negative in Ovariectomized SD Rat;** • **Negative in F-344 Rat,** • **Negative in 3 Strains of Mice** • **SD Rat Prone to Develop Mammary Tumors due to Accelerated Reproductive Aging.** • **Established Mode of Action Not Relevant to Humans** • **Carcinogenic Response Not Observed in SD rat below 3.5 mg/kg/day**

10.0 References

1. Proposed Guidelines for Carcinogen Risk Assessment. United States Environmental Protection Agency, Office of Research and Development, April, 1996.

2. Mayhew, A.D., 1986. Two year chronic feeding/oncogenicity study in rats administered atrazine, American Biogenics Corporation, Project No. 410-1102. American Biogenics Corporation, April 29, 1986. MRID No. 00158930.

3. Thakur, A. K., 1992. Oncogenicity Study in Sprague-Dawley Rats with Atrazine Technical. Project No. 483-275. Hazleton Washington, January 27, 1992. MRID No. 42204401.

4. Thakur, A. K., 1991. Determination of hormone levels in Sprague-Dawley rats treated with atrazine technical, Hazleton Laboratories, Vienna, VA, Project No. 483-278. Hazleton Washington, October 17, 1991. MRID No. 42085001.

5. Thakur, A. K., 1992. Oncogenicity Study in Fischer-344 Rats with Atrazine Technical. Project No. 483-277. Hazleton Washington, February 18, 1992. MRID No. 42227001.

6. Thakur, A. K., 1991. Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical. Project No. 483-279. Hazleton Washington, November 8, 1991. MRID No. 42146101.

7. Pinter, A., Torok, G., Borzsonyi, M., Surjan, A., Csik, M., Kelecsenji, Z., Kocsis, Z., Long-term carcinogenicity bioassay of the herbicide atrazine in F-344 rats. Neoplasma, 37(5), 533-544, (1990).

8. Statistical Report for Survival and Mammary Tumor Analyses from the Fischer 244/Lati Rat Study Analyses. Covance Laboratory Study Number 6117-998, September 2, 1999, MRID Number. 44917701.

9. US EPA. 1999. Atrazine: Review of an atrazine carcinogenicity study in the open literature. US EPA, November 29, 1999.

10. Morseth, S.L., 1998. Chronic (12-24 Month) Study in Rats with Atrazine Technical. Covance Laboratory Study Number 2386-108. April 15, 1998.

11. Spindler, M. and Summer, D., 1981. Two-Year Chronic Oral Toxicity Study with Technical Atrazine in Albino Rats. Project No. 622-06769. Industrial Bio-Test Laboratories, January 15, 1981. MRID No. 00089151.

12. Rudzki, M. W., McComick, G. C. and Arthur, A. T., 1991. Atrazine - Chronic toxicity study in rats (derived from 2-gen.), Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, NJ. Project No. MIN 852214, January 28, 1991.

13. Petterson, J. C.; Turnier, J. C. 1995. One-Year chronic toxicity study with atrazine technical in rats, Environmental Health Center, Farmington, USA, Rep. No. F-00171, December 8, 1995. MRID No. 43598623.

14. Sumner, D.D., Carcinogenicity Study with Atrazine Technical in Albino Mice. Project No. 8580-8906. Industrial Bio-Test Laboratories, June 30, 1981. MRID No. 00085399.

15. Hazelette, J.R., Green, J.D., 18-Month Oncogenicity Study of Atrazine Technical in Mice. Project No. MIN 842120. Ciba-Geigy Pharmaceuticals SEF, October 30, 1987. MRID No. 40431302.

16. Innes, J.R.M., et al. 1969. Chronic Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note. J. Nat. Canc. Inst. 42, 1101-1114,

17. Stevens, J.T., Breckenridge, C.B., Wetzel, L., Gilles, JH., Luempert III, L., and Eldridge, J.C. 1994. Hypothesis for Mammary Tumorigenesis in Sprague-Dawley Rats Exposed to Certain Triazine Herbicides. Journal of Toxicology and Environmental Health, 43, 139-153.

18. Stevens, J.T., Breckenridge, C.B., Wetzel, L., Thakur, A.K., Werner, C. Luempert III, L., and Eldridge, J.C. 1999. A Risk Characterization for Atrazine: Oncogenicity Profile. Journal of Toxicology and Environmental Health, Part A., 56, 69-109.

19. Hazelette, J.R., Green, J.D., Ametryn 104-Week Oral Toxicity/Oncogenicity Study in Rats. Project MIN 842119 Ciba-Geigy Pharmaceuticals, SEF, August 24, 1987 MRID No. 40349906.

20. Chau, R.Y., McCormick, G.C., Arthur, A.T., 104-Week Oral Toxicity/ Carcinogenicity Study of Prometryn Technical in Rats. Project No. MIN 872225. Ciba-Geigy Pharmaceuticals, SEF, March 4, 1991. MRID No. 41901201

21. Jessup, D.C., Terbutryn Technical 2-Year Chronic Oral Toxicity Study in Rats. Project No. 382-008, IRDC, October 4, 1979 MRID No. 40356901(EPA ACC. No. 242570-71)

22. O'Conner, D.J., McCormick, G.C., Green, J.D., 104-Week Oral Chronic Toxicity and Carcinogenicity Study of Prometon in Rats. Project No. MIN 852003. Ciba-Geigy Pharmaceuticals SEF, January 14, 1988. MRID No. 40488102.

23. Rolofson, G.L., Two-Year Chronic Oral Toxicity Study with GS-14259 Technical (Terbumeton) in Albino Rats. Industrial Bio-Test Laboratories, November 15, 1981.

24. Lamoureux, G.L., Simoneaux, B., and Larson, J. 1998. The Metabolism of Atrazine and Related 2-chloro-4,6-bis(alkylamino)-s-triazines in Plants. In: L. Ballantine, J.E Mcfarland and D. Hackett, eds. Triazine Herbicides: Risk Assessment. ACS Symposium Series No. 683, ACS, pp 60-81.

25. Chow, E., Emeigh-Hart, S.G. 1994. 2-Year Dietary Chronic Toxicity/Oncogenicity Study with G-34048 Technical in Rats. Project No. F-00125. Ciba-Geigy Environmental Health Center, June 22, 1994. MRID No. 43532001.

26. Wu, J., Robinson, R.A. and Simoneaux, B. 1998. Metabolism of Selected (s)-Triazines in Animals. In: L. Ballantine, J.E. McFarland, and D. Hackett, eds., Triazine Herbicides Risk Assessment. ACS Symposium Series No. 683, pp. 104-113.

27a Davidson, I.W.F, 1988; Metabolism and kinetics of atrazine in man. Project No. 101947, Bowman Gray School of Medicine, MRID No. 43598603.

27b Cheung, M.W., 1990, Analysis of Human Urine to determine Residues of Atrazine, G-28273, G-28279, and G-30033 Resulting from Oral Ingestion of Atrazine Including Storage Stability Results, Ciba-Geigy Corp. Agricultural Division, Residue Chem. Department, Greensboro, NC 27410, USA, Rep. N° ABR-90034, March 7, 1990, MRID No. 43598604.

28 Hamboeck, H., Fischer, R. W., Di Iorio, E. E., Winterhalter, K. H. 1981 The Binding of s-Triazine Metabolites to Rodent Hemoglobins Appears Irrelevant to Other Species. Molecular Pharmacology, 20, 579-584,

29. Sathiakumar, N., E. Delzell, and P. Cole. 1995. Mortality among workers at two triazine manufacturing plants. Am. J. Ind. Med. 29:143-151.

30. Loosli, R. Epidemiology of Atrazine. 1995, Reviews of Environmental Contamination and Toxicology, 143, 47-57.

30a. Sathiakumar, N. and E. Delzell. 1997. A review of epidemiologic studies of triazine herbicides and cancer. Critical Reviews in Toxicology 27:599-612.

30b. Delzell, E., Sathiakumar, N., MacLennan, P. 2000. A follow-up study of mortality among workers at the Novartis St. Gabriel plant. Final report. June 12, 2000 (unpublished).

31. Neuberger, J.S. 1996. Atrazine and/or triazine herbicides exposure and cancer: an epidemiologic review. J. Agromedicine 3:9-30.

32. Cantor, K.P., A. Blair, G. Everett, R. Gibson, L.F. Burmeister, L.M. Brown, L. Schuman, and F.R. Dick. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res. 52:2447-2455.

33. Hoar, S.K., A. Blair, F.F. Holmes, C.D. Boysen, R.J. Robel, R. Hoover, and J. F. Fraumeni, Jr. 1986. Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma. J. Am. Med. Assoc. 256:1141-1147.

34. Zahm, S.H., D.D. Weisenburger, P.A. Babbitt, R.C. Saal, K.P. Cantor, and A. Blair. 1988. A case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska (abstract). Am. J. Epidemiol. 127:901.

35. Zahm, S.H., D.D. Weisenburger, K.P. Cantor, F.F. Holmes, and A. Blair., 1993a. Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma. Scand. J. Work Environ. Health 19:108-114.

36. Zahm, S.H., D.D. Weisenburger, R.C. Saal, J.B. Vaught, P.A. Babbitt, A.Blair. 1993b. The role of agricultural pesticide use in the development of non-Hodgkin's lymphoma in women. Archives of Environmental Health 48:353-358.

36a. Kogevinas, M., Kauppinen, T., Winkelmann, R., Becher, H., Bertazzi, P.A., Bueno-de-Mesquita, H.B., Coggon, D., Green, L., Johnson, E., Littorin, M., Lynge, E., Marlow, D.A., Mathews, J.D., Neuberger, M., Benn, T., Pannett, B., Pearce, N., Saracci, R. 1995. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. Epidemiology, 6:396-402.

36b. Clavel, J., Hemon, D., Mandereau, L., Delemotte, B., Severin, F., Flandrin, G. 1996. Farming, pesticide use and hairy-cell leukemia. Scand. J. Work Environ. Health, 22:285-293.

37. Brown, L.M., L.F. Burmeister, G.D. Everett, and A. Blair. 1993. Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control 4:153-156.

38. Burmeister, L.F. 1990. Cancer in Iowa farmers: recent results. Amer. J. Ind. Med.18:295-301.

39. Brown, L.M., A. Blair, R. Gibson, G.D. Everett, K.P. Cantor, L.M. Schuman, L.F Burmeister, S.F. Van Lier, and F. Dick. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer res. 50: 6585-6591.

40. Hoar, S.K., A. Blair, F.F. Holmes, C. Boysen, and R.J. Robel. 1985. Herbicides and colon cancer. Lancet 1:1277-1278.

41. Blair, A. and Zahm, S. H.. Cancer among farmers., Occup. Med. State of the Art Reviews, 6, 335-354, 1991.

42. Blair, A., Zahm,S.H., Pearce, N.E., Heinman, E.F., and Fraumeni, J.F., Jr., Clues to cancer etiology from studies of farmers. Scand. J. Work Environ. Health. 18, 209-215, 1992.

43. Donna, A., P. Betta, F. Robutti, P. Crosignani, F. Berrino, and D. Bellingeri. 1984. Ovarian mesothelial tumors and herbicides: a case-control study. Carcinogenesis 5: 941-942.

44. Donna, A., P. Crosignani, F. Robutti, P.G. Betta, R. Bocca, N. Mariani, F. Ferrario, R. Fissi, and F. Berrino. 1989. Triazine herbicides and ovarian epithelial neoplasms. Scand. J. Work Environ. Health 15:45-53.

44a. Crosignani, P., Donna, A., Berrino, F. 1990. Authors' reply to letter. Scand. J. Work Environ. Health, 16:446-447.

45. Kettles, M., A., Browning, S. R., Prince, T.S., and Horstman, S.W. Triazine Herbicide Exposure and Breast Cancer Incidence: An Ecologic Study of Kentucky Counties. Environmental Health Perspectives, 105 (11), 1222-1227, 1997.

46. Mills, P.K., Correlation Analysis of Pesticide Use Data and Cancer Incidence Rates in California Counties. Archives of Environmental Health, 1998, 53(6), 410-413.

47. Van Leeuwen, J.A, Waltner-Toews, D., Abernathy, T., Smit, B., & Shoukri, M., Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987-1991. International Journal of Epidemiology, 1999, 28, (5), 836-840.

48. Brusick, D. J. 1994, An assessment of the genetic toxicity of atrazine: Relevance to health and effects. Mut. Res., 317, 133-144

49. US EPA. 1998. ENVIRONMENTAL PROTECTION AGENCY[OPPTS-42206; FRL-6021-3]Endocrine Disruptor Screening Program AGENCY: Environmental Protection Agency (EPA). Federal Register: August 11, 1998 63, 154: 42852-42855.

50. Tennant, M. K., Hill, S. D., Eldridge, J. C., Wetzel, T. L., Breckenridge, C. B. and Stevens, T. J. 1994. Chloro-s-triazines antagonism of estrogen action: interaction with estrogen receptor binding. J. Toxicol. Environ. Health, 43(2), 197-211.

51. Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciarretta, C., Safe, S., Zacharewski, T. 1996. Failure of chloro-S-triazine-derived compounds to induce estrogen receptor-mediated responses in vivo and in vitro. Fund. Appl. Toxicol., 30, 93-101.

52. Tennant, M. K., Hill, S. D., Eldridge, J. CH., Wetzel, T. L., Breckenridge, Ch. B. and Stevens, T. J. 1994. Possible antiestrogenic properties of chloro-s-triazines in rat uterus. J. Toxicol. Environ. Health, 43(2), 183-196.

53. Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Serrano, F.O., 1995, The E-SCREEN Assay as a Tool to Identify Estrogens: An Update on Estrogenic Environmental Pollutants. Environ. Health Perspect. 103 (Suppl.7), 113-122 (1995).

54. Tran, D.Q., Kow, K.Y., McLachlan, J.A., Arnold., S.F. 1996. The inhibition of estrogen receptor-mediated responses by chloro-S-triazine-derived compounds is dependent on estradiol concentration in yeast. Biochem. Biophys. Res. Comm., 227, 140-146.

55. Graumann, K., Briethofer, A., and Jungbaur, A. 1999. Monitoring of estrogen mimics by recombinant yeast assay; synergy between natural and synthetic compounds? Sci. Tot. Environ. 225: 69-79.

56. McConnell, R.F. A histomorphologic reevaluation of the ovaries, uterus, vagina, mammary gland, and pituitary gland from Spraque-Dawley and Fischer 344 female rats treated with atrazine. MRID Report # 43598622, 1995.

57. Giknis, M.L.A., Atrazine technical, A Teratology (Segment II) Study in Rats. Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division Summit, New Jersey, USA, Unpublished report No. 882049, 23.02.1989.

58. Arthur, A.T., 1984, A teratology study of atrazine in New Zealand White rabbits. Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division Summit, New Jersey, USA, Unpublished report No. 832110, 13.09.1984.

59. Mainiero, J.; Youreneff, M.; Giknis, M.L.A., Yau, E.T., 1987, Atrazine technical - Two-generation reproduction study in rats. Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division Summit, New Jersey, USA, Unpublished report No. 852063, 17.11.1987.

60. Cooper, R.L., Stoker, T.E., Goldman, J.M., Hein, J., and Tyrey, G. Atrazine disrupts hypothalamic Control of Pituitary - Ovarian Function. The Toxicologist, 30(1): 66, 1996.

61. Cooper, R.L., M.B. Parrish, W.K. McElroy, G.L. Rehnberg, J.F. Hein, J.M. Goldman, T.E. Stoker and L. Tyrey, Effects of Atrazine on the Hormonal Control of the Ovary. The Toxicologist, 15(1), Abstract #1572, 1995.

62. Tyrey, L., Stoker, T., Hein, J. and Cooper, R. 1996. Atrazine suppression of luteinizing hormone secretion in the rat. Program of the U.S. Environmental Protection Agency Symposium on Susceptibility and Risk, Durham.

63. Morseth, S.L., 1996. Evaluation of the Luteinizing Hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats - Interim report, Corning Hazleton Inc., Vienna, VA, USA, Rep. No. CHV 2386-111, January 25, 1996.

64. Simpkins, J.W., Eldridge, J.C., and Wetzel, L.T. 1998. Role of Strian-Specific Reproductive Patterns in the Appearance of Mammary Tumors in Atrazine-Treated Rats. In: L. Ballantine, J.E Mcfarland and D. Hackett, eds. Triazine Herbicides: Risk Assessment. ACS Symposium Series No. 683, ACS, pp. 399-413.

65. Morseth, S.L., 1996. Evaluation of the Luteinizing Hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats - 6-Months report, Corning Hazleton Inc., Vienna, VA, USA, Rep. N° CHV 2386-111, October 25, 1996.

66. Sielken, R.L., Valdez-Flores, C.,and Holden, L.,1999 Palpable Tumors in Sprague-Dawley Rats: Time to Tumor Analyses., JSC Sielken, October 27, 1999, pp 1- 54.

67. Simpkins, J.W. , Relevance of the Female Sprague-Dawley (SD) Rat for Human Risk Assessment of Chloro-s-Triazines., January 11, 2000, pp. 1 – 12.

68. Yoshida, H., Suzuki, M., Okugawa, K., Wada, S., Fukunsiii, R, Okamoto, S., and Matsumoto, K., Mammary Carcinoma Induced by a Series of Intragastric Intubations of 7,12-Dimethylbenz[a]anthracene in Gonadectomized Female and Male Sprague-Dawley Rats. 1982, Gann, 73, 539-542.

69. Clinton, S.K., Li, P., S., Mulloy, A.L., Imrey, P.B., Nandkumar, S., and Visek, W.J., 1995, The Combined Effects of Dietary Fat and Estrogen on Survival, 7-12-Dimethylbenz [a] anthracene-induced Breast Cancer and Prolactin Metabolism in Rats., 1995, American Institute of Nutruition, 1192-1204.

70. Gullino, P.M., Pettigrew, H.M., and Grantham, F.H., N-Nitrosmethylurea and Mammary Gland Carcinogen in Rats. 1975, Journal National Cancer Institute, 54(20), 401-414.

71. Simpkins J.W., Eldridge J.C., and Wetzel L.T. 1998. Role of Strain-Specific Reproductive Patterns in the Appearance of Mammary Tumors in Atrazine Treated Female Rats In: L. Balantine, J.E. McFarland, and D. Hackett, Triazine Herbicides Risk Assessment, ACS Symposium Series No. 683, pp. 399-413.

72. Eldridge J.C, McConnell R.F, Wetzel L.T, and Tisdel M.O. 1998. Appearance of Mammary Tumors in Atrazine-Treated Female Rats: Probable Mode of Action Involving Strain-Related Control of Ovulation and Estrous Cycling. In: Triazine Herbicides Risk Assessment. In: L. Balantine, J.E. McFarland, and D. Hackett, Triazine Herbicides Risk Assessment, ACS Symposium Series No. 683, pp. 414–423.

73. Cooper, R.L., Goldman, J.M. and Stoker, T.E. 1999. Neuroendocrine and reproductive effects of contemporary-use pesticides. Toxicol. Ind. Health 15: 26-36.

74. Cutts, H. J., Noble, R.L. 1964. Estrone-induced Mammary Tumors in the Rat. Cancer Research, 24: 1116-1123.

75. McConnell, RF. Comparative aspects of contraceptive steroids: effects observed in rats. *Toxicol Pathol*, 1989, 17(2):385-388.

76. Banerjee SK; De A; Sarkar DK. 1994. Colocalization of prolactin and proliferating cell nuclear antigen in the anterior pituitary during estrogen-induced pituitary tumors. Cancer Lett 87(2):139-44.

77. Arita J; Kojima Y; Kimura F. 1989. Enhanced dopamine synthesis and release in vitro in the median eminence of rat hypothalamus are associated with involution of estradiol-induced pituitary tumors. Endocrinology 124(4):1998-2004.

78. Sarkar DK, Gottschall PE, Meites J. 1982. Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. Science 12;218(4573):684-686.

79. Welsch C.W., Jenkins T.W., Meites J. 1970. Increased Incidence of Mammary Tumors in the Female Rat Grafted with Multiple Pituitaries. Cancer Research, 30: 1024 – 1029.

80. Meites, J. 1972. Relation of Prolactin and Estrogen to Mammary Tumorigenesis in the Rat. J National Cancer Institute, 48 (4): 1217-1224.

81. Hauswirth, J. W. and Wetzel, L.T. 1998. Toxicity Characteristics of the Chlortriazines: Atrazine and Simazine. In: Triazine Herbicides Risk Assessment. In: L. Balantine, J.E. McFarland, and D. Hackett, Triazine Herbicides Risk Assessment, ACS Symposium Series No. 683, pp.370- 383.

82. Sawyer CH First Geoffrey Harris Memorial Lecture. Some recent developments in brain-pituitary-ovarian physiology. Neuroendocrinology 17:97-124,1975.

83. Simpkins, J.W., J.P. Advis, C.A. Hodson and J. Meites. Blockade of steroid induced LH release by selective depletion of anterior hypothalamic norepinephrine activity. Endocrinology 104, 506-509, 1979a.

84. Simpkins, J.W., H.H. Huang, J.P. Advis, and J. Meites. Evaluation of changes in NE and DA turnover during progesterone induced LH and prolactin surges in ovariectomized, estrogen primed rats. Biology of Reproduction 20, 625-632, 1979b.

85. Wise PM, Kashon ML, Krajnak KM, Rosewell KL, Cai A, Scarbrough K, Harney JP, McShane T, Lloyd, JM, Weiland NG Aging of the female reproductive system: a window into brain aging. Recent Prog Horm Res 52:279-303, 1997.

86. Simpkins, J.W., Millard, W.J., Gabriel, S.M., Soltis, E.E., Noradrenergic methods in Neuroendocrinology. In Handbook of Pharmacologic Methodologies for the Study of the Neuroendocrine System. Eds. R.W. Steger, A Johns, CRC Press, Boca Raton, FL. 1985, pp. 1-63.

87. Ordog T, Goldsmith JR, Chen MD, Connaughton MA, Hotchkiss J, Knobil E On the mechanism of the positive feedback action of estradiol on luteinizing hormone secretion in the rhesus monkey. J Clin Endocrinol Metab 83:4047-4053, 1998.

88. Krey LC, Butler WR, Knobil E Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. Endocrinology 96:1073-87, 1975.

89. Goodman RL, Knobil E The sites of action of ovarian steroids in the regulation of LH secretion. Neuroendocrinology 32:57-63,1981.

90. Pohl CR, Knobil E The role of the central nervous system in the control of ovarian function in higher primates. Annu Rev Physiol 44:583-593,1982.

91. Weiss, G. Schmidt, C., Kleinberg, D.L. Ganguly,M. Positive feedback effects of oestrogen on LH secretion in women in neuroleptic drugs. Clinical Endocrinol. 7: 423-427, 1977.

92. Knobil, E. On the control of gonadotrophin secretion in the Rhesus monkey, Recent Prog. Hormone Res. 30: 1-36, 1974.

93. Aschheim, P., Aging in the hypothalamic-hypophyseal ovarian axis in the rat. In Hypothalamus, pituitary and Aging, Ed. A. V. Everitt and J. A. Burgess, Charles C. Thomas Company, Springfield, Il, 1976, pp. 376-418.

94. Meites, J., H.H Huang , J.W. Simpkins. Recent studies on neuroendocrine control of reproductive senescence in rats. In The Aging Reproductive System, Ed. Edwiard L. Scheider, Raven Press, New York, 1978, pp. 213-235.

95. Schiff, I., Wilson, E, Clinical Aspects of aging of the female reproductive system. In The Aging Reproductive System, Ed. Edwiard L. Scheider, Raven Press, New York, 1978, pp. 9-28.

96. Taylor, A. E., Polycystic ovary syndrome. Endocrinol Metab Clin North Am. 27: 877-902, 1998.

97. Shelley., D. R., Dunaif, A., Polycystic ovary syndrome. Compr. Ther. 16:26-34, 1990.

98. Knab, D. R., Estrogen and endometrial carcinoma. Obstet Gynecol. Surv. 32: 267-281, 1977.

99. Cheung, A. P., Lu, J. K., Chang., R. J., Pulsatile gonadotrophin secretion in women with polycystic ovary syndrome after gonadotrophin-releasing hormone agonist treatment. Hum Reprod. 12:1156-1164, 1997.

100. Dennefors, B. L., Knutson, F., Janson, P. O., Jansson, L., Hamberger, L., Ovarian steroid production in a woman with polycystic ovary syndrome associated with endometrial cancer. Acta Obstet Gynecol Seand. 64: 387-392, 1985.

101. Barnes, R. B., Diagnosis and therapy of hyperandrogenism. Baillieres Clin Obstet Gynaecol. 11:369-396, 1997.

102. Hall, J. E., Taylor, A. E., Hayes, F. J., Crowley, W. F., Jr. Insights into hypothalamic-pituitary dysfunction in polycystic ovary syndrome. J. Endocrinol Invest. 21:602-611, 1998.

103. Marshall, J. C., Neuroendocrine aspects of polycystic ovary syndrome. Endocrinal Metab Clin North Am. 28:295-324, 1999.

104. Arroyo, A., Laughlin, G. A., Morales, A. J., Yen, S. S., Inappropriate gonadotropin secretion in polycystic ovary syndrome: influence of adiposity. J. Clin Endocrinol Metab. 82: 3728-3733, 1997.

105. Minanni S. L., Marcondes, J. A., Waichenberg, B. L., Cavaleiro A. M., Fortes, M. A., Rego, M. A., Vezozzo, D. P., Robard D. Giannella-Neto D., Analysis of gonadotropin pulsatility in hirsute women with normal menstrual cycles and in women with polycystic ovary syndrome. Fertil Steril. 71:675-683, 1999.

106. Hayes, F. J., Taylor, A. E., Martin, K. A., Hall, J. E. Use of a gonadotropin-releasing hormone antagonistic as a physiologic probe in polycystic ovary syndrome: assessment of neuroendocrine and androgen dynamics. J. Clin Endocrinol Metab. 83:2343-2349, 1998.

107. Halmi, K.A. The state of research in anorexia nervosa and bulimia. Psychiatr Dev 1983 Autumn: 1(3):247-62.

108. Gandar, R. Collin, D. Functional hypothalamic amenorrheas.J. Gynecol Obstet Biol Reprod. 1993b:22(2):133-40.

109. Kiningham, R.B., Apgar, B.S., Schwenk, T.L. Evaluation of amenorrhea. Am Fam Physician. 1996.Mar:53(4):1185-94.

110. Magiakou, M.A., Mastorakos, G., Webster, E., Chrousos, G.P.. The hypothalamic-pituitary-adrenal axis and the female reproductive system. Academy Science 1997 Jun 17:816:42-56.

111. Rogol, A.D., Weltman, J.Y., Evans, W.S., Veldhuis, J.D., Weltman, A.L. Long-term endurance training alters the hypothalamic-pituitary axes for gonadotropins and growth hormones. Endocrino Meta Clin North Am. 1992 dec:21 (4):817-32.

112. Bhasin, S., Swerdloff, R.S. Hypothalamic hypodonadism. Spec Top Endocrinol Meta 1985:7:237-66.

113. Marshall, J.C., Dalkin, A.C. Haisenleder, D.J., Griffin, M.L., Kelch, R.P. GnRH pulses-the regulators of human reproduction.Trans Am Clin Climatol Assoc. 1992;104:31-46.

114. Santoro, N., Filicori, M., Crowley, W.F. Jr. Hypogonadotropic disorders in men and women; diagnosis and therapy with pulsatile gonadotropin-releasing hormone. Encocr Rev 1986 Feb:7(1)11-23.

115. Gandar, R., Collin, D. Functional hypothalamic amenorrheas. J. Gynecol Obstet Biol Reprod. 1993a:22(2)127-32.

116. Legan, S.J. and Karsch, F. J. Daily signal for the LH surge in the rat. Endocrinology 96: 57-62, 1975.

117. IARC Monographs Evaluations of Atrazine (CAS 1912-2409) and Simazine (CAS 122-34-9). Volume 73. In press. Expected publication 2/00.

118. National Registration Authority review of Atrazine. November, 1997. Reviewed by National Registration Authority for Agricultural and Veterinary Chemicals of Australia. Canberry Australia.

119. Delzell, E., Mandel, J., & Breckenridge, C. A review of the epidemiological studies on atrazine and other chlorotriazine herbicides. July 19, 2004

120. Laws, S. C. Ferrell, J.M., Stoker, T.E., & Cooper, R.L., 2003 Pubertal development in female Wistar rats following exposure to propazine, and atrazine biotransformation products, diamino-s-chlorotriazine and hydroxyatrazine. Toxicological Sciences, 76, 190-200.

121. United States Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division. The grouping of as series of triazine pesticides based on a common mechanism of toxicity. March, 2002.

122. United States Environmental Protection Agency, Atrazine Toxicology Chapter of the Re-registration Eligibility Decision, Second Revision, April 11, 2002.

123. Sanderson JT, Seinen W, Giesy JP, van den Berg M. 2000. 2-Chloro-*s*-Triazine Herbicides Induce Aromatase (CYP19) Activity in H295R Human Adrenocortical Carcinoma Cells: A Novel Mechanism for Estrogenicity? *Toxicol. Sci.* 54: 121-127.

124. Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M. 2001. Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. *Environ Health Perspect* 109(10): 1027-31

125. Sanderson, JT, Boerma J, Lansbergen GW, van den Berg M. 2002. Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. *Toxicol. Appl Pharmacol.* 182 (1): 44-54

126. Heneweer, M., van den Berg, M. Sanderson, J.T., 2004 A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase. Toxicology Letters, 146-183-194.

127. Sasano H, Harada N. 1998. Intratumoral Aromatase in Human Breast, Endometrial, and Ovarian Malignancies. *Endocrine Reviews* **19**(5): 593–607.

128. Hanioka N, Jinno H, Tanaka-Kagawa T, Nishimura T, Ando M. 1998a. Changes in Rat Liver Cytochrome P450 Enzymes by Atrazine and Simazine Treatment. *Zenobiotica* 28(7): 683-698.

129. Stoker TE, Laws SC, Guidici DL, Cooper RL. 2000. The effect of atrazine on puberty in male Wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol Sci* 58(1):50-9.

130. Modic W, Ferrell J, Wood C, Laskey J, Cooper R, Laws S. 2004. Atrazine alters steroidogenesis in male Wistar Rats. *Toxicologist.* Abstract No. 568.

131. Rayner JL, Wood C, Fenton SE. 2004. Exposure parameters necessary for delayed puberty and mammary gland development in Long–Evans rats exposed in utero to atrazine. *Toxicol Appl Pharmacol.* 195: 23– 34.

132. Kazeto Y, Place AR, Trant JM. 2004. Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. Aquatic Toxicology 69: 25–34.

133. Hecker M, Coady KK, Villeneuve DL, Murphy MB, Jones PD, Geisy JP. Response of *Xenopus Laevis* to Atrazine Exposure: Assessment of the Mechanism of Action of Atrazine. Aquatic Toxicology Lab, Michigan State University. ECORISK NumberMSU-04. February 13, 2003.

134. Crain DA, Guillette LJ Jr, Rooney AA, Pickford DB. 1997. Alterations in steroidogenesis in alligators (Alligator mississippiensis) exposed naturally and experimentally to environmental contaminants. *Environ Health Perspect*. 105(5):528-33.

135. Covance, 2004. Oral gavage study on the effect of atrazine on pituitary hormone secretion of ovariectomized, estrogen-replaced female Rhesus monkeys. July 14, 2004.

136. Terasawa, E. & Fernandez. D.L. 2001, Neurobiological Mechanisms of the Onset of Puberty in Primates Endocrine Reviews, 22(1), 111-151

137. Velduis, J.D., 1996. Neuroendocrine mechanisms mediating awakening of the human gonadotropic axis in puberty. Pediatric Nephrology, 10, 304-317.

Bibliography of Documents Submitted by Syngenta Crop Protection for the Atrazine and Simazine Special Review (Updated July 19, 2004

Summary Documents (All Issues)

Chapter 1: Summary of Comments on the Special Review Position Document 1 For Pesticide Products Containing Atrazine and Simazine. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 22: Summary of Additional Comments on the Response to the Special Review Position Document 1 for Pesticide Products Containing Atrazine and Simazine, Supplement I. Submitted January 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934401).

Additional Research Planned by Ciba-Geigy Corporation: Draft Protocols. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 23: Summary of Additional Comments on the Response to the Special Review Position Document 1 for Pesticide Products Containing Atrazine and Simazine, Supplement 2 (Supplement to EPA MRID No. 43934401) Submitted October 30, 1996 to Public Docket OPP -0000-60. (EPA MRID No. 44152101).

Chapter 24: Letter from Thomas J. Parshley, Novartis Crop Protection, Inc. Summarizing Data Submission Dated June 30, 1997. Submitted June 30, 1997 to Public Docket OPP-30000-60.

Chapter 25: Letter from Thomas J. Parshley, Novartis Crop Protection, Inc. Summarizing Data Submission Dated June 26, 1998. Submitted June 26, 1998 to Public Docket OPP-30000-60.

Overview of Atrazine Monitoring Program, Ecomonitoring and Watersheds in Mitigation. Provided on a Compact Disk (CD). Submitted July 19, 2004.

Benefits

Chapter 4: Benefits/Alternatives Summary for Atrazine and Simazine. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 5: Farming Trends and Practices. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 6: Sustainable Agriculture: The Potential of Non-Chemical Weed Control Methods as Substitutes for Herbicides in U.S. Corn Production. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 7: Environmental Benefits of Atrazine and Simazine. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 8: Role of Atrazine and Simazine in Weed Resistance Management. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 9: Economic Evaluations: Lost Benefits From Atrazine or Triazine Herbicide Cancellations (U.S. Impact). Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 10: Economic Review of Past Atrazine and Triazine Herbicide Assessments. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 11: Comparative Analysis of Alternatives in Corn. Submitted March 23, 1995 to Public Docket OPP-30000-60. (Resubmitted October 9, 1996)

Chapter 12: Comparative Analysis of Alternatives in Sorghum. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 13: Benefits of Atrazine in Ecofallow. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 14: Benefits of Atrazine in Sugarcane, Macadamia Nuts, and Guava. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 15: Benefits of Atrazine and Simazine in Turf and Ornamental Nurseries. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 16: Benefits of Atrazine and Simazine in Conifers. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 17: Benefits of Simazine in Fruit and Nuts. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 18: Benefits of Simazine in Citrus. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 19: Supporting Market Research Documentation. Submitted March 23, 1995 to Public Docket OPP-30000-60.

The Role of Atrazine in Managing Weed Bio-Types Resistant to ALS-Inhibitor Herbicides. Submitted to EPA Public Docket OPP-30000-60 October 30, 1996.
MRID No. 44152127

Supplement to Ciba's Benefits Analysis of Atrazine and Simazine. 2 Volumes. Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934422).

Supplement to Novartis' Benefits Analysis of Atrazine and Simazine - Additional Information on Weed Control, Yield and Impact to Growers and Livestock Producers, Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315417).

Syngenta's Comments on the EPA's November 20, 2000 Draft "Atrazine: HED's Preliminary Human Health Risk Assessment (and Associated Documents) for the Reregistration Eligibility Decision (RED)." Syngenta Crop Protection, Inc. Submitted December 22, 2000.

Ecological Effects

Ecological Risk Assessment of Atrazine In North American Surface Waters. Atrazine Ecological Risk Assessment Panel (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60. 3 Volumes (EPA MRID No. 43598639).

Ecological Risk Assessment of Atrazine in North American Surface Waters: (Environmental Toxicology and Chemistry, Volume 15, Number 1, 1996.). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934419).

Ecological Risk Assessment of Atrazine in North American Surface Waters: Additional Considerations of Exposure and Ecological Effects. (Expert Panel Report.). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934420).

Characterization of *Selenastrum capriocornutum* Response to Episodic Atrazine Exposure (Study No. 09524). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934421).

Simazine Aquatic Exposure and Risk Assessment. Peters, 1995. Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598644).

Discussion of Published Reports Concerning Effects of Simazine on Aquatic Life. (1995) Submitted May 23, 1995 to Public Docket OPP-30000-60. (EPA MRID No. 43659601).

1996 Panel Report: Beyond the Probabilistic Approach: Risk Assessment of Atrazine in North American Surface Waters (Supplement to EPA MRID No. 43934419) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152126).

Comments on the Reregistration Eligibility Science Chapter for Atrazine: Environmental Fate and Effects Chapter. Syngenta Crop Protection, Inc. Submitted January 9, 2001

Aquatic Ecological Risk Assessment of Atrazine - A Tiered Probabilistic Approach: A Report of an Expert Panel. Study No. 709-00. Submitted January 8, 2001 (EPA MRID No. 45299501)

Exposure Assessment of Atrazine in Surface Waters: A Tiered Probabilistic Modeling Approach: Study No. 376-01. Submitted January 8, 2001 (EPA MRID No. 45299502)

A Risk-Based Assessment of Endocrine System Responses in Fish, Amphibians, and Reptiles to Atrazine. Study No. 710-97. Submitted January 8, 2001 (EPA MRID No. 45299503)

Effects of Atrazine on the Sex Ratio of *Daphnia pulicaria*, Study No. 45810. Submitted January 8, 2001 (EPA MRID No. 45299504).

Summary of Environmental Fate of Atrazine. Study No. 1213-99. Submitted January 8, 2001 (EPA MRID No. 45299505).

Response to the EPA Request for Additional Information for the Aqueous Photolysis of 14C-Atrazine Under Natural and Artificial Light. Study No. 534-95. Submitted Nov. 20, 2001. (EPA MRID No. 45545301.)

Determination of Potential Effects of 10 Day Neonatal Exposure of Atrazine on Histological and Hormonal Sex Determination in Incubated American Alligator (Alligator Mississipiensis) Eggs. Study No. 1244-98. Submitted Nov. 20, 2001. (EPA MRID No. 45545302).

Determination of Potential Effects of 10 Day Neonatal Exposure of Atrazine on Histological and Hormonal Sex Determination in Incubated Red-Eared Slider (Psuedemys Elegans) Eggs. Study No. 1245-98. Submitted Nov. 20, 2001. (EPA MRID No. 45545303)

Supplement to "Aquatic Ecological Risk Assessment of Atrazine - A Tiered Probabilistic Approach", Including Responses to EPA Comments. Study No. 709-00 Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622302)

Response to the EPA Document Entitled "Review of Atrazine PRA". Study No. 1274-02. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622303)

Determination of Potential Effects of 20-Day Exposure of Atrazine on Endocrine Function in Adult Largemouth Bass (*Micropterus Salmoides*). Study No. 1168-90. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622304)

Endocrine System Responses in Fish, Amphibians, and Reptiles to Atrazine: Assessment of an Expert Panel (Study No. 1725-02). Submitted July 3, 2002. (EPA MRID NO 45711301)

Atrazine Technical SF-Toxicity to Midge (Chironomus tentans) Under Flow-Through Conditions. Study No. 1500-02. Submitted April 8, 2002. (EPA MRID No. 45904001)

Atrazine Technical SF-Toxicity to Midge (Chironomus tentans) During 10-Day Sediment Exposure. Study No. 1501-02. Submitted April 8, 2002. (EPA MRID No. 45904002)

Field Exposure of Xenopus Laevis to Atrazine and Other Triazines in South Africa: Exposure Characterization and Assessment of Laryngeal and Gonadal Responses. Study No. 109-02. Submitted February 27, 2003. (EPA MRID No. 45867701)

Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (Rana Clamitans) Collected from Agricultural Areas in Michigan: Hormone Analysis. Study No. 1163-02. Submitted February 27, 2003. (EPA MRID No. 45867702)

A Pilot Study of Response of Larval Rana Clamitans to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology and Selected Hormones and Enzyme Activities. Study No. 1164-02. Submitted February 27, 2003. (EPA MRID No. 45867703)

Response of Xenopus Laevis to Atrazine Exposure: Assessment of the Mechanism of Action of Atrazine. Study No. 1165-02. Submitted February 27, 2003. (EPA MRID No. 458677004)

Histology of the Gonads and Analysis of Hormone Levels in the Native Bull Frog (Rana Catespiena) Collected form Agricultural Areas in Southern Iowa: Pilot Study. Study No. 1311-02. Submitted February 27, 2003. (EPA MRID No. 458677005)

Characterization of Atrazine Exposures and Potential Effects in Florida ecosystems Dominated by Sugarcane Agriculture; A reconnaissance Survey of Amphibians in South Florida for the Assessment of Potential Atrazine Effects. Study No. 1312-02. Submitted February 27, 2003. (EPA MRID No. 458677006)

Response of Larval Xenopus Laevis to Atrazine Exposure: Assessment of Metamorphosis and Gonadal and Laryngeal Morphology. Study No. 1833-01. Submitted February 27, 2003. (EPA MRID No. 45867707)

Methods Development for the Study of Mechanism of Action of Atrazine in Adult and Metamorphosing Xenopus Laevis and Rana Clamitans: Aromatase Induction. Study No. 1834-01). Submitted February 27, 2003. (EPA MRID No. 45867708)

Field Exposure of Xenopus Laevis to Atrazine and Other Triazines in South Africa: Feasibility Study for Site Characterization and Assessment of Laryngeal and Gonadal Responses. Study No. 2011-02. Submitted February 27, 2003. (EPA MRID No. 45867709)

Gonadal and Laryngeal Responses to Field Exposure of Xenopus Laevis to Atrazine in Areas of Corn Production in South Africa. Study No. 2012-02. Submitted February 27, 2003. (EPA MRID No. 45867710)

Exposure of Xenopus Laevis Larvae to Different Concentration of Atrazine in Semi-Natural Microcosms. Study No. 2233-02. Submitted February 27, 2003. (EPA MRID No. 45867711)

South African Analytical Support - Hormone and Aromatase Analysis. Study No. SA-01C. Submitted February 27, 2003. (EPA MRID No. 45867501).

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Overview. Study No. 2318-03. Submitted September 15, 2003. (EPA MRID No. 46078601)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Summary of EPA EFED ecological Risk Assessment. Study No. 2319-03. Submitted September 15, 2003. (EPA MRID No. 46078602)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: National Risk Assessment for Aquatic Organisms and Critical Habitat Atrazine Endangered Species Risk Assessment Series. Study No. 2320-03. Submitted September 15, 2003. (EPA MRID No. 46078603)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: national Risk Analysis for Terrestrial Organisms and Critical Habitat Atrazine Endangered Species Risk Assessment Series. Study No. 1913-02. Submitted September 15, 2003. (EPA MRID No. 46078604)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: National Risk Analysis Summary of Results. Study No. 2322-03. Submitted September 15, 2003. (EPA MRID No. 46078605)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Spatial Scoping Analysis for All Uses. Study No. 2323-03. Submitted September 15, 2003. (EPA MRID No. 46078606)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Agricultural Usage Data Analysis. Study No. 2324-03. Submitted September 15, 2003. (EPA MRID No. 46078607)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Site Specific Analysis. Study No. 2325-03. Submitted September 15, 2003. (EPA MRID No. 46078608)

Information on the Potential Impact of Atrazine Uses on Federally Listed Threatened and Endangered Species: Taxonomic Specific Analysis. Study No. 2326-03. Submitted September 15, 2003. (EPA MRID No. 46078609)

Atrazine Technical: A 14-Day Static-Renewal Toxicity Text with Duckweed (Lemna Gibba G3) Including a Recovery Phase. Study No. 1450-03. Submitted December 11, 2003. (EPA MRID No. 46150901)

Endocrine Responses of Amphibians to Atrazine: Update Assessment of an Expert Panel. Study No. 1725-02. Submitted May 30, 2003. (EPA MRID No. 46007501).

Human Health Risk Assessments

Preliminary Risk Characterization for Atrazine and Simazine (Sielken Project No. 56). 3 Volumes. Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934415).

Chapter 2 Part A: Evaluation of the Carcinogenic Potential of Atrazine and the Relevance to Human Risk Assessment Part B: Ecological Risk Assessment of Atrazine in North America's Surface Waters. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Chapter 3 Part A: Evaluation of the Carcinogenic Potential of Simazine and the Relevance to Human Risk Assessment Part B: Ecological Risk Assessment of Simazine in North America's Surface Waters. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Weight of Evidence on the Oncogenic Potential of Atrazine: Consensus Panel Report, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60. (EPA MRID No. 43598624).

Weight of Evidence on the Oncogenic Potential of Simazine: Consensus Panel Report. (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60. (EPA MRID No. 43598640).

Weight of Evidence on the Oncogenic Potential of Atrazine: Consensus Panel Report. Submitted October 30, 1996 to Public Docket OPP-30000-60. EPA MRID No. 44152105. (This volume replaces MRID No. 43598624)

Risk Characterization for Atrazine and Simazine. (Sielken Project No. 56) (Replaces EPA MRID No. 43934415) 3 Volumes. Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152125).

Atrazine: Report and Proposed Decision of the United Kingdom Made to the European Commission Under Article 7(1) of Regulation 3600/92 Council Directive 91/414/EEC Regulation 3600/92 3 Volumes. Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315415).

Simazine: Report and Proposed Decision of the United Kingdom Made to the European Commission Under Article 7(1) of Regulation 3600/92 Council Directive 91/414/EEC Regulation 3600/92 3 Volumes. Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315416).

The NRA Review of Atrazine: Existing Chemical Review Program, National Registration Authority for Agriculture and Veterinary Chemicals of Australia; November 1997, 3 Volumes. Submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597607).

International Agency for Research on Cancer published report for the World Health Organization. Volume 73 Pages 59-113 Atrazine. 1999.

International Agency for Research on Cancer published report for the World Health Organization. Volume 73 Pages 625-640, Simazine. 1999.

Human Hazard (Toxicity/Mode of Action)

2-Year Dietary Chronic Toxicity/Oncogenicity Study with G-34048 in Rats: Lab Study No. F00125 (1995). Submitted January 31, 1995 to Public Docket OPP-30000-60. 6 Volumes. (EPA MRID No. 43532001).

An Assessment of the Genetic Toxicity of Atrazine: Relevance to Human Health and Environment Effects. Brusick, (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598601).

Teratological Evaluations of Atrazine Technical, a Triazine Herbicide, in Rats and Rabbits. Infurna et. al., (1984). Submitted March 23, 1995 to Public Docket OPP-30000-60. (EPA MRID No. 43598607).

Hypothesis for Mammary Tumorigenesis in Sprague-Dawley Rats Exposed to Certain Triazine Herbicides. Stevens et. al., (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60. (EPA MRID No. 43598611).

Factors Affecting Mammary Tumor Incidence in Chlorotriazine-Treated Female Rats: Hormonal Properties, Dosage and Strain. Eldridge et. al., (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598612).

Rat Mammary Tumorigenesis: Relevance of Hormonal Imbalance to Dose Selection. Stevens, (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598613).

Short-Term Effects of Chlorotriazines on Estrus in Female Sprague-Dawley and Fischer 344 Rats. Eldridge et. al., (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598614).

Chronic Effects of Atrazine on Estrus and Mammary Tumor Formation in Sprague-Dawley and Fischer 344 Rats. Wetzel et. al., (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598615).

Ultrastructural Changes in Rat Hypothalamic Arcuate Nucleus Following Long-Term Diaminochlorotriazine Feeding. Tennant et. al., (1993) Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598616).

Possible Antiestrogenic Properties of Chloro-s-Triazines in Rat Uterus. Tennant et. al., (1994a). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598617).

Chloro-s-Triazine Antagonism of Estrogen Action: Limited Interaction with Estrogen Receptor Binding. Tennant et. al., (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598618).

Failure of Atrazine and Simazine to Induce Estrogenic Responses in MCF-7 Human Breast Cancer Cells. Safe et. al., (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598619).

Evaluation of a Hormonal Mechanism for Mammary Carcinogenesis of the Chlorotriazine Herbicides: Consensus Panel Report; Simpkins (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598620).

Ciba Response to EPA's May 3, 1994, EPA Letter by Penelope Fenner Crisp, Director, Health Effects Division, Office of Prevention, Pesticides and Toxic Substances U.S., EPA, Wetzel and Tisdel, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598621).

A Histomorphologic Reevaluation of the Ovaries, Uterus, Vagina, Mammary Gland, and Pituitary Gland from Sprague-Dawley and Fischer-344 Female Rats Treated with Atrazine. McConnell, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598622).

Preliminary Report: 1-Year Chronic Toxicity Study with Atrazine Technical in Rats. Study No. F-00171. Turnier et. al., (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598623).

1-Year Chronic Toxicity Study With Atrazine Technical In Rats (Study No. F-00171). Submitted January 31, 1996 to Public Docket OPP-30000-60. 2 Volumes (EPA MRID No. 43934402).

Failure of Chloro-S-Triazine Derived Compounds to Induce Estrogenic Responses In Vivo and In Vitro. (Environmental Toxicity, 1996, In Press). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934403).

Evaluation of the Luteinizing Hormone (LH) in Atrazine-Exposed Female Sprague-Dawley Rats - Pilot Study (Study No. CHV 2386-109). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934404).

Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats - Method Validation (Study No. CHV 2386-110). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934405).

Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats (Study No. CHV 2386-111). Submitted January 31, 1996 to Public Docket OPP-30000-60. 2 Volumes (EPA MRID No. 43934406).

Atrazine Technical: Chronic Toxicity Study in Rats. (Study No. 852214) 2 Volumes Submitted April 4, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44005301)

Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats - 6 Month Study (Study No. CHV 2386-111). 2 Volumes Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152102)

Chronic (12/24 Month) Study with Atrazine Technical (Study No. CHV 2386-108) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152103).

Evaluation of a Hormonal Mode of Action for Mammary Carcinogenesis: Second Consensus Panel (Supplement to EPA MRID No. 43598620) Submitted October 30, 1996 to Public Docket OPP-30000-60.(EPA MRID No. 44152104)

Induction of Estradiol, 2-Hydroxylase Activity in MCF-7 Human Breast Cancer Cells by Pesticides and Carcinogens. Safe - Accepted for Publication in Environmental Toxicology and Pharmacology. Submitted to EPA June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315402).

Evaluation of a Hormonal Mode of Action for Mammary Carcinogenesis of the Chloro-s-Triazine Herbicides: Third Consensus Panel Report Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315401).

Chronic (12-24 Month) Study in Rats with Atrazine Technical (Ovariectomized Rats): Study No. 2386-108. Submitted April 27, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44544701)

Atrazine Mode of Action Bridging Studies for Simazine and DACT: Rationale and Draft Protocols; Submitted April 16, 1998nmto Public Docket OPP-30000-60. (EPA MRID Nos. not assigned - protocols).

Supplemental Information for Atrazine Mode of Action: Bridging Studies for Simazine and DACT (Rationale & Draft Protocols) Submitted December 9, 1998 to EPA Public Docket OPP-30000-60. (EPA MRID No. 44713801)

3-Month Oral Toxicity Study in Rats Submitted December 9, 1998 to EPA Public Docket OPP-30000-60. (EPA MRID No. 44723701)

The Absorption, Distribution, Degradation and Excretion of (U-14C) Triazine G-30027 in the Rat. Submitted Decmeber 9, 1998 to EPA Public Docket OPP-30000-60. (EPA MRID No. 44713802)

Statistical Report for Survival and Mammary Tumor Analyses From the Fischer 344/Lati Rat Study (Pinter et al., 1990). Submitted to EPA Public Docket OPP-30000-60 September 2, 1999 (EPA MRID No. 44917701

Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT Via Oral gavage for One Month. Submitted to EPA Public Docket OPP-30000-60 on March, 2000. MRID No. 45058701

Atrazine Effects on Testosterone and Androgen-Dependent Reproductive Organs in Peripuertal Male Rats. Submitted to EPA Public Docket OPP-30000-60, March, 2000. MRID No. 45058702.

An Update to the October 7, 1999 Question/Reference Requests for Atrazine. Compact Disk containing 248 files at 86 MB. Submitted October 28, 1999 to EPA Public Docket OPP-30000-60.

Summary of Atrazine's Mode of Action in the Female Sprague-Dawley Rat. Submitted October 28, 1999 to EPA Public Docket OPP-30000-60.

Palpable Tumors in Sprague-Dawley Rats: Time-to-Tumor Analyses. Sielken. Submitted October 28, 1999 to EPA Public Dockett OPP-30000-60.

Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine, or DACT for Six Months; Statistical Analysis of the LH Surge. Study No. 2214-01. Submitted August 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45471001).

Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine, or DACT Vian Oral Gavage for One Months. Study No. 1198-98. Submitted August 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45471002).

52-Week Toxicity Study of Simazine, Atrazine, and DACT Administered in the Diet to Female Rats. Study No. 2214-01. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622309)

Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine, or DACT Vian Oral Gavage for Six Months: Statistical Analysis of the LH Surge: Supplemental Analysis. Study No. 2214-01. Submitted March 12, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45629402)

The Effects of Atrazine on the Sexual Maturation of Female Alderley Park-Wistar and Sprague-Dawley Rats . Study No. 1775-02. Submitted July 3, 2002. EPA MRID No. 45722401)

Effects of Atrazine on the First Spontaneous Ovulation of Female SD Rats Administered Pregnant Mare's Serum Gonadotropin (PMDG) on PND 30. Study No. 1755-02. Submitted July 3, 2002. EPA MRID No. 45711303)

Oral (Gavage) Study on the Effect of Atrazine on Pituitary Hormone Secretion of Ovariectomized, Estrogen; Replaced Female Rhesus Monkeys. Syngenta Study No. T002160-03. Submitted July 19, 2004. (EPA MRID No. not yet assigned.)

Human Exposure (Water)

Human Exposure to Atrazine and Simazine via Ground and Surface Drinking Water. Clarkson, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60. 3 Volumes (EPA MRID No. 43598634).

Atrazine Exposure Through Drinking Water: Exposure Assessments for Ohio, Illinois, and Iowa. Richards, et. al., (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598635).

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water: Update I (Supplement to MRID No. 43598634). 4 Volumes. Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934413).

Ciba/State Ground-Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States (Study No. 174-91) 24 Volumes. Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934414).

Ciba/State Ground-Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States-Amendment to the Final Report, EPA MRID No. 43934414; Contract Lab No. 242.01. Submitted February 20, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44222601)

Ciba/State Ground-Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States-Second Amendment to the Final Report, EPA MRID No. 43934414; Contract Lab No. 242.01. Submitted February 20, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44222602)

Ciba/State Ground-Water Monitoring Study for Simazine and Its Major Degradation Products in the United States (Study No. 151-92). 10 Volumes. Submitted July 2, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44049201).

Ciba/State Ground-Water Monitoring Study for Simazine and Its Major Degradation Products in the United States-Amendment to the Final Report, EPA MRID No.44049201; Contract Lab No. 242.02. Submitted February 20, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44222603)

Ciba/State Ground-Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States-Second Amendment to the Final Report, EPA MRID No. 44049201; Contract Lab No. 242.02. Submitted February 20, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44222604)

Retrospective Shallow Subsurface Water Monitoring Study for Atrazine and Its Major Metabolites on Sod Production Sites in Florida (1996). 2 Volumes. Submitted April 2, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 43973001).

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water: Update II (Supplement to MRID No. 43598634) 7 Volumes Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152122).

Summary of Voluntary Atrazine Monitoring Program at Selected Community Water Systems (Study No. ABR-96102) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152123).

Summary of Sampling Methods for Estimating Annual Mean Concentrations of Atrazine in Community Water Systems. (Report No. ABR-96103) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152124).

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water: Update III (Supplement to EPA MRID No. 44152122) 7 Volumes submitted June 30, 1997 to EPA Public Docket OPP-30000-60. (EPA MRID No. 44315414).

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water: Update IV (Supplement to EPA MRID No.44315414) 6 Volumes. Submitted June 30, 1998 to EPA Public Docket OPP-30000-60. (EPA MRID No. 44597601).

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water: Update IV ;Supplement to EPA MRID No 44597601; Cumulative and Community Water Systems (CWS) Population Exposure Distributions for Atrazine and Simazine; Project No. 00300 Submitted to EPA Public Docket OPP-30000-60. (EPA MRID No. 44711001)

Atrazine Annual Maximum and Mean Concentrations at CWS In 21 Major Use States, PLEX database, 1993-1998. Supplement to EPA MRID No. 44597601. 7 Volumes. Submitted to EPA Public Docket OPP-30000-60 December 13, 1999.

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water; Update V. 7 volumes submitted to EPA OPP-30000-60 March 2, 2000. MRID No.45058704.

Human Exposure to Atrazine and Simazine Via Ground and Surface Drinking Water; Update VI. 10 volumes submitted to EPA OPP-30000-60 October, 2000. MRID No.45253401

Novartis/Community Water System Surface Water Monitoring Study for Atrazine and Its Major Degradation Products in Seven States in the United States. Submitted to EPA Public Docket OPP-30000-60 March, 2000.

Novartis /Community Water System Surface Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States. Study No. (95) (419-97,117213) Submitted March 2, 2000 to EPA Public Docket OPP 30000-60. MRID No. 45058703.

Novartis/Community Water System Surface Water Monitoring Study for Atrazine and Its Major Degradation Products in the United States. Supplementary Report - Regression Analysis. Submitted June 15, 2000 to EPA Public Docket OPP-30000-60. MRID No. 45145601

Response to EPA Questions About Regression Analyses for Water Monitoring Data in Several States (EPA MRID No. 45145601). Submitted September 14, 2000 to EPA Public Docket OPP 30000-60. MRID No. 45209601

Syngenta/Community Water System Ground Water Monitoring Study for Atrazine and Its Major Degradation Products in Multiple States in the United States. Study No. 758-00. Submitted May 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45399906).

Atrazine and Total Chloro-Triazine Concentrations (TCT) from Community Water Systems (CWS) on Surface water in 31 Atrazine Major Use States from 1993-1999. Study No. 1264-99. Submitted August 9, 2001 to EPA Public Docket OPP 30000-60. MRID No. 45475101

Atrazine and Total Chloro-Triazine Concentration (TCT) for Raw and Finished Water from Community Water Systems (CWS) on Surface Water that Participated in the Syngenta Voluntary Monitoring Program,1993-2000. Study No. 1265-99. Submitted August 9, 2001 to EPA Public Docket OPP 30000-60. MRID No. 45475102

Probabilistic Assessment of Drinking Water and Dietary Exposure Combined. Study No. 2214-01. Submitted to EPA OPP 30000-60 September 21, 2001. MRID No. 45503101

Total Chlorotriazine Probabilistic Water Exposure Assessment Using Calendex. Study No. 2314-01. Submitted to EPA OPP 30000-60 September 21, 2001. MRID No. 45503102.

Resampling of Domestic Rural Drinking Water Wells. Study No. 1697-01. Submitted Nov. 20, 2001. (EPA MRID No. 45545304)

Storage Stability of Atrazine, G-30033, G-26279, and G-28273 in Water Under Refrigerator Storage Conditions. Study No. 105-91. Submitted Nov. 20, 2001. (EPA MRID No. 45545305)

Trend of Atrazine Concentrations Monitored in Ohio Lake Erie Tributary Drainage basins and Community Water Systems (CWS) on Surface Water of Six States. Study No. 1662-01. Submitted Nov. 20, 2001. (EPA MRID No. 45545306).

Exposure Analysis of Atrazine and Simazine in Community Water Systems in 32 Use States, 1993-2000: Update VII. Study No. 1629-00. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622305)

Probabilistic Assessment of Drinking Water and Dietary Exposure Combined Using Water Concentration Data Between 1993 and 2001. Study No. 1305-02. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622306)

Analysis of Time Trends in Total Chlorotriazine Residue Concentrations in Finished Water in Twenty-Eight Community Water Supply Systems Between 1993-2001. Study No. 1292-02. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622307)

Long Term (1994-2000) Trend Analysis of Atrazine Concentrations from 127 Midwestern Water Bodies. Study No. 2694-01. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622308).

Probabilistic Assessment of Drinking water and Dietary exposure Combined Using Calendax Based on Water Concentration Data Between 1993-2001. Study No1365-02. Submitted March 12, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45629401)

The Selection of Endpoints, Application of FQPA Uncertainty Factors, and Risk Extrapolation at the 99.9 Percentile . Study No. 1776-02. Submitted July 3, 2002. EPA MRID No. 45711302)

Occurrence of Atrazine in Community Water Systems on Groundwater and Rural Wells in High Atrazine Use Areas. Study No. 1778-02. Submitted July 3, 2002. EPA MRID No. 45711304)

An Analysis of the Trends in Atrazine Concentration in Raw and Finished Drinking Water in High Atrazine Use Areas. Study No. 1777-02. Submitted July 3, 2002. EPA MRID No. 45711305)

An Analysis of the Trends in Total Analysis of Chlorotriazine Residue Concentrations in Finished Water in 28 Community Water Supply Systems Between 1993-2001 (Amendment 1). (MRID No. 45622307).Study No. 1292-02). Submitted July 3, 2002. EPA MRID No. 45711306)

Effects of Time Trends in Total Chlorotriazine Residue Concentrations in Finished Water in 28 Community Water Supply Systems Between 1993-2001 (Amendment 2). (MRID No. 45622307). Study No. 1292-02). Submitted July 3, 2002.EPA MRID No. 45711306)

Probabilistic Assessment of Exposure to Total Chlorotriazines in Drinking Water Using Calendex: 11 Additional Sites. Study No. 1757-02. Submitted July 3, 2002. EPA MRID No. 45711308).

A Statistical-Based Ratinale for Identifying vulnerable Community Water Systems (CWS) Delivering Drinking Water Derived from Surface Water. Submitted December 23, 2002. (EPA MRID No. 45824501).

Comparison of 90-Day Rolling Averages to Annual Means of Quarterly Sampled Data. A Monte Carlo Simulation. Study No. 2203-02. Submitted April 8, 2003. (EPA MRID No. 45904003).

Magnitude, Duration, Temporal Occurrence Frequency of Atrazine Residues in North American Waters. Study No. 1745-02). Submitted April , 2003. (EPA MRID No. 45904004).

Atrazine Monitoring Program - Selection of Candidate Community Water Systems to Participate in the 2003 Program. Study No. 1287-03. Submitted March 18, 2003. (EPA MRID No. 45882901)

2001 Safe Drinking Water Act Data for Atrazine and Simazine. Study No. 1913-03. Submitted September 5, 2003 (EPA MRID No. 46083001)

2002 Safe Drinking Water Act Data for Atrazine and Simazine. Study No. 1914-03. Submitted September 5, 2003 (EPA MRID No. 46083002)

Atrazine Watershed Mitigation Plan for Old Lake and Lake George-Marion Community Water System Marion, Kentucky. Study No. 1788-03. Submitted July 30, 2003. (EPA MRID No. 46046901).

Atrazine Watershed Mitigation Plan for Lake Ellis and Lake Morris Chariton Community Water System Chariton, Iowa. Study No. 1789-03. Submitted July 30, 2003. (EPA MRID No. 46046902).

Atrazine Watershed Mitigation Plan for Spa Lake-Lewisburg Community Water System Lewisburg, Kentucky. Study No. 1790-03. Submitted July 30, 2003. (EPA MRID No. 46046903).

Atrazine Watershed Mitigation Plan for Upper Terrebonne Basin- Iberville Water District #3 Community Water System Iberville, Louisiana. Study No. 1791-03. Submitted July 30, 2003. (EPA MRID No. 46046904).

Atrazine Watershed Mitigation Plan for Bucklin Lake- Bucklin Community Water System Bucklin, Missouri. Study No. 1792-03. Submitted July 30, 2003. (EPA MRID No. 46046905).

Atrazine Watershed Mitigation Plan for Dearborn Lake-Dearborn Community Water System Dearborn Missouri. Study No. 1793-03. Submitted July 30, 2003. (EPA MRID No. 46046906).

Atrazine Watershed Mitigation Plan for Bischoff, Oser and Hahn Reservoirs-Batesville Water and Gas Utility Community Water System Batesville, Indiana. Study No. 1794-03. Submitted July 30, 2003. (EPA MRID No. 46046907).

Atrazine Watershed Mitigation Plan for Gillespie Lakes-Gillespie Community Water System Gillespie, Illinois. Study No. 1795-03. Submitted July 30, 2003. (EPA MRID No. 46046908).

Bischoff, Oser, and Hahn Reservoirs Watershed Mitgation Plan 2003 Fourth Quarter Report Batesville Water and Gas Utility Community Water System Batesville, Indiana. Study No. 1794-03. Submitted December 23, 2003. (EPA MRID No. 46161801).

Bucklin Lzke Watershed Atrazine Mitigation Plan 2003 Fourth Quarter Report Bucklin Community Water System Bucklin, Missouri. Study No. 1792-03. Submitted December 23, 2003. (EPA MRID No. 46161802).

Lake Ellis and Lake Morris Watershed Atrazine Mitigation Plan 2003 Fourth Quarter Report Chariton Community Water System Chariton, Iowa. Study No. 1789-03. Submitted December 23, 2003. (EPA MRID No. 46161803).

Gillespie Lakes Watershed Atrazine Mitigation Plan 2003 Fourth Quarter Report Gillespie Community Water System Gillespie, Illinois. Study No. 1795-03. Submitted December 23, 2003. (EPA MRID No. 46161804).

Upper Terrabonne Basin Watershed Atrazine Mitigation Plan 2003 Fourth Quarter Report Iberville water District #3 Community Water System Iberville, Louisiana. Study No. 1791-03. Submitted December 23, 2003. (EPA MRID No. 46161805).

Old Lake and Lake George Watersheds Atrazine Mitigation Plan 2003 Fourth Quarter Report Marion Community Water System Marion, Kentucky. Study No. 1788-03. Submitted December 23, 2003. (EPA MRID No. 46161806).

2003 Atrazine Monitoring Program Report. Study No. 1301-03. Submitted January 30, 2004. (EPA MRID No. 46184501).

Atrazine Feasibility Study. Study No. 1500-03. Submitted February 3, 2004. (EPA MRID No. 46190301).

Old Lake and Lake George Watersheds Atrazine Mitigation Plan Watershed Stakeholder Mitigation Meeting April 29, 2004 Marion, Kentucky; (T001788-03). (EPA MRID No. not yet assigned.)

Lake Ellis and Lake Morris Watershed Atrazine Mitigation Plan Watershed Stakeholders Mitigation Meeting March 15, 2004 Chariton, Iowa; (T001789-03). (EPA MRID No. not yet assigned.)

Upper Terrebonne Basin Watershed Atrazine Mitigation Plan Watershed Stakeholder Mitigation Meeting January 06, 2004 Port Allen,Louisiana; (T001791-03). (EPA MRID No. not yet assigned.)

Bucklin Lake Watershed Atrazine Mitigation Plan Watershed Stakeholder Mitigation Meeting March 30, 2004 Bucklin,Missouri; (T001792-03). (EPA MRID No. not yet assigned.)

Bischoff, Oser, and Hahn Reservoirs Watersheds Atrazine Mitigation Plan Watershed Stakeholder Mitigation Meeting March 26, 2004 Batesville, Indiana; (T001794-03). (EPA MRID No. not yet assigned.)

Gillespie Lakes Watershed Atrazine Mitigation Plan Watershed Stakeholder Mitigation Meeting,March 24, 2004 Gillespie, lllinois; (T001795-03). (EPA MRID No. not yet assigned.)

Water Stewardship

Chapter 20: Product Stewardship Actions and Environmental Protection Programs to Address Worker Exposure and Drinking Water Exposure. Submitted March 23, 1995 to Public Docket OPP-30000-60.

Summary of Best Management Practices to Reduce (Mitigate) Runoff of Atrazine into Surface Water. Ciba-Geigy Corporation, ABR-95045, Balu, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598636).

Best Management Practices for Water Quality: 1996 Update Submitted October 30, 1996 to Public Docket OPP 30000-60. (EPA MRID No. 44152128)

Validation Study of an Atrazine Immunoassay for Drinking Water Monitoring in Compliance with the Safe Drinking Water Act. Submitted June 11, 1999 to Public Docket OPP 30000-60. (Replaces EPA MRID No. 44769801)

Human Exposure (Diet)

Nature of Atrazine and Simazine Metabolism in Animals and Plants. Ciba-Geigy Corporation, ABR-95039. Simoneaux, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598602).

^{14}C Atrazine: Nature of the Residue in Sugarcane, Supplement No. 1, Lab. Project ID HWI 6117-181, MRID No. 43016503. Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598628).

^{14}C Atrazine: Nature of the Residues in Corn and Sorghum, Amendment No. 2, Lab. Project ID HWI 6117-178, MRID No. 42547116. Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598629).

Atrazine: Magnitude of Residues in Field Corn Forage, Silage-Stage Forage, Fodder, and Grain and Sweet Corn Ears and Forage Following Applications of AAtrex® 4L to Field Corn and Sweet Corn, ABR-91070, B. Gold, (1995a). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598630).

Atrazine: Magnitude of Residues in Grain Sorghum Forage, Hay, Silage-Stage Forage, Fodder, Grain, and Processed Grain Fractions Following Applications of AAtrex® 4L to Grain Sorghum, ABR 91071, B. Gold, (1995b). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598631).

Atrazine and Metolachlor - Magnitude of Residues in Soil and Grain Sorghum Following Applications of AAtrex® 4L and Dual® 8E With and Without the Addition of Acrysol ("G-

110"). ABR-93080, Selman, (1995a). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598632).

Dietary Risk Exposure Assessment for Atrazine. Ciba-Geigy Corporation, ABR-94021, Wurz, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598633).

Dietary Risk Exposure for Simazine. Ciba-Geigy Corporation, ABR-94022, Wurz, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598641).

Updated Dietary Exposure Assessment for Atrazine (ABR-96009). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934411).

Determination of Transfer Rate and Nature of Residue(s) in Milk from ^{14}C-Atrazine Treated Cows (Study No. CHW 6117-325). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934412).

^{14}C-Atrazine: Nature of the Residues in Corn and Sorghum (MRID 42547116), Amendment No. 3 to the Final Report; Study No. HWI 6117-178, Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152119).

^{14}C-Atrazine: Nature and Magnitude of the Hydroxytriazine and Chlorotriazine Residues in Sorghum Following a Preemergence Application at 2 lb. ai/A (Study No. CHW 6117-337) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152120)

^{14}C-Atrazine: Nature and Magnitude of the Hydroxytriazine and Chlorotriazine Residues in Corn Following a Preemergence Application at 2 lb ai/A (Study No. CHW 6117-335) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152121)

Atrazine: Magnitude of the Residues In or On Corn (Study No. ABR-96087) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152117).

Atrazine: Magnitude of the Residues In or On Grain Sorghum (Study No. ABR-96088) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152118)).

Revised Dietary Exposure Assessment for Atrazine (ABR-96105) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152115).

Updated Dietary Exposure Assessment for Simazine (ABR-96093) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152116).

Summary of the Nature and Magnitude of Atrazine Residues on Corn and Sorghum Grain, Forage, and Fodder and the Basis for the Dietary Risk Assessment. Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315406).

Revised Dietary Assessment for Atrazine Including Two Exposure Scenarios (ABR-97064). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315407).

Validation of Analytical Method AG-484A for the Determination of Residues of Atrazine, G-30033, G-28279, and G-28273 in Corn and Sorghum (ABR-97056). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315412).

Rationale for the Dairy Cattle Diet Utilized in the Revised Dietary Exposure Assessment for Atrazine and Simazine. Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315413).

Atrazine: Magnitude of the Residues in or on Corn - Amendment 1 (EPA MRID No. 44152117). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315410).

Atrazine: Magnitude of the Residues in or on Sorghum - Amendment 1 (EPA MRID No. 44152118). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315411).

^{14}C-Atrazine: Nature and Magnitude of the Hydroxytriazine and Chlorotriazine Residues in Corn Following a Preemergence Application at 2 lb. ai/A - Amendment No. 2 to the Final Report (EPA MRID No. 44152121). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315408).

^{14}C-Atrazine: Nature and Magnitude of the Hydroxytriazine and Chlorotriazine Residues in Sorghum Following a Pre-emergence Application at 2 lb. ai/A - Amendment No. 2 to the Final Report (EPA MRID No. 44152120). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315409).

Atrazine: Magnitude of the Residues in or on Corn - Amendment 2 (EPA MRID No. 44152117). Submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597602).

Atrazine: Magnitude of the Residues in or on Sorghum - Amendment 2 (EPA MRID No. 44152118). Submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597603).

Atrazine: Corn Supporting Data for Amending Tolerances. Study No. 827-99. Submitted May 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45399901).

Atrazine: Sorghum Supporting Data for Amending Tolerances. Study No. 826-99. Submitted May 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45399902).

Atrazine: Chronic Dietary Exposure Assessment for Atrazine and the Simazine Metabolites Common to Atrazine. Study No. 1256-00. Submitted May 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45399904).

Atrazine: Field Accumulation in Rotational Crops. Study No. 169-99. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622301)

Human Exposure (Workers)

Analysis of Human Urine to Determine Residues of Atrazine, G-28273, G-28279, and G-30033 Resulting from Oral Ingestion of Atrazine, Including Storage Stability Results. Cheung, (1990). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598604).

Metabolism and Kinetics of Atrazine in Man. Davidson, (1988). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598603).

The In Vitro Percutaneous Absorption of Formulated [U^{14}C]-Triazine G-30027 (Atrazine) and [U^{14}C]-Triazine G-27692 (Simazine) Through Human and Rat Abdominal Epidermis. Jack, (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598606).

Summary of Assessment of Worker Exposure for Atrazine in Response to the U.S. Environmental Protection Agency Issuance of "The Triazine Herbicides Position Document 1 Initiation of Special Review." Ciba-Geigy Corporation, ABR-95041, Selman, (1995a). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598637).

Assessment of Worker Exposure for Atrazine in Response to the U.S. Environmental Protection Agency Issuance of "The Triazine Herbicide Position Document 1 Initiation of Special Review." ABR-95038, Selman, (1995b). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598638)

Summary of Assessment of Worker Exposure for Simazine in Response to U.S. Environmental Protection Agency Issuance of The Triazine Herbicide Position Document 1: Initiation of a Special Review. ABR-95042, Selman, (1995c). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598642).

Assessment of Worker Exposure for Simazine in Response to U.S. Environmental Protection Agency's Issuance of The Triazine Herbicide Position Document 1: Initiation of a Special Review. ABR-95030, Selman, (1995d). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598643).

Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration - Interim Report (Study No. 95SU04). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934407).

Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration - Interim Report (ABR-95131). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934408).

In Vivo Percutaneous Absorption of Atrazine In Man - Interim Report (Study Number H832-11835-01). Submitted January 31, 1996 to Public Docket OPP-30000 60 (EPA MRID No. 43934409).

In Vivo Percutaneous Absorption of Atrazine In Man - Interim Report (ABR-96003). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934410).

Analytical Method for the Semi-Quantitative Determination of Atrazine Mercapturate in Urine by Enzyme Immunoassay Including Validation Study (Analytical Method No. AG-638). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934416).

Evaluation of the Potential Exposure of Workers to Atrazine During Commercial Mixing, Loading, and Spray Application to Corn (EPA Subpart U) - Biological Field Phase (Study No. 95-501HE). 2 Volumes . Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934417).

Assessment of Potential Worker Exposure to Atrazine During Commercial Mixing, Loading, and Application to Corn (ABR-95133). Submitted January 31, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 43934418).

Disposition of Atrazine in Rhesus Monkey Following Intravenous Administration (Study Nos. UCSF-95SU04, BDH-081-1, ABR-96066, ABR-96073) Submitted October 30, 1996 to Public Docket OPP-30000-60 (EPA MRID No. 44152112).

Disposition of Atrazine in Rhesus Monkey Following Oral Administration (Study No. 95SU01, ABR-96094). Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152113).

In Vivo Percutaneous Absorption of Atrazine in Man (Study Nos. H832-11835-01, BDH-08-2, ABR-96067, ABR-96073). Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152114).

Evaluation of the Potential Exposure of Workers to Atrazine During Commercial Mixing, Loading, and Spray Application to Corn (EPA Subpart U) - Biological Field Phase (Study No. 95-501HE). 2 Volumes. Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152111).

Evaluation of Potential Worker Exposure to Atrazine during Commercial Mixing, Loading, and Application to Corn (Supplement to Report No. ABR-95133, MRID No. 43934418) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152109).

Analytical Method for the Determination of Residues of Atrazine, G-30033, G-28279, and G-28273 in Human Urine by Gas Chromatography/Mass Selective Detection Including Validation Data, (Analytical Method No. AG-637) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152110).

An Updated Assessment of Worker Exposure for Atrazine in Response to the U.S. Environmental Protection Agency Issuance of the "Triazine Herbicide Position Document 1 Initiation of Special Review" (Study No. ABR-96071) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152106).

An Updated Assessment of Worker Exposure for Simazine in Response to the U.S. Environmental Protection Agency Issuance of the "Triazine Herbicide Position Document 1 Initiation of Special Review" (Study No. ABR-96072) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152107).

Supplemental Data and Evaluation of Exposure to Lawn Care Operators Using Atrazine in the Southern United States (Report No. ABR 96069; Supplement to ABR-95038, MRID No. 43598638) Submitted October 30, 1996 to Public Docket OPP-30000-60. (EPA MRID No. 44152108).

Disposition of Atrazine in Rhesus Monkey Following Oral Administration (ABR-96094) - Final Report Amendment 1 (EPA MRID No. 44152113) submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315405).

Assessment of Potential Worker Exposure to Atrazine During Commercial Mixing, Loading, and Application to Corn - Amendment 1 (EPA MRID No. 43934418). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315403).

Presentation of Data from ABR-95133 "Assessment of Potential Worker Exposure to Atrazine During Commercial Mixing, Loading, and Application" for Use in the Pesticide Handler's Exposure Database (PHED 1.1). Submitted June 30, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44315404).

Evaluation of the Potential Internal Dose of Atrazine to Workers During Mixing-Loading and Application of Atrazine Products - Biological Field Phase - (Study No. 95-502HE) 2 Volumes. Submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597606).

Evaluation of the Potential Internal Dose of Atrazine to Workers During Mixing-Loading and Application of Atrazine Products - Biological Monitoring- ABR-97094 (Study No. 179-95) submitted June 30, 1998 to Public Docket OPP-30000-60. (EPA MRID No. 44597605).

Comparison of Exposure Assessments to Atrazine and Simazine for Commercial Operators and Farmers Who Mix, Load, and/or Apply Atrazine (ABR 98068). Submitted June 30, 1998 to Public Docket OPP-30000-60 (EPA MRID No. 44597604).

Atrazine Impregnation on Dry Bulk Fertilizer and Mixing Atrazine with Liquid Bulk Fertilizer: A Description of the Processes and Occupational Risk Assessment. Study No. 1273-01. Submitted May 2, 2001 to Public Docket OPP-30000-60. (EPA MRID No. 45399905).

Atrazine Use in Southern Turf: Additional Information for Residential Turf Risk Assessments. Study No. 1143-01. Submitted to Public Docket OPP-30000-60 October 10, 2001. (EPA MRID No. 45517301)

Determination of Dermal Transfer Efficiency of Granular Atrazine Residues form Turf to Dry and Wetted Palms. Study No. 1841-01. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622310)

Children's Residential Exposure and Risk Assessment to Atrazine Treated Turf Using Hand Press Transfer Efficiency Data. Study No. 1255-02. Submitted February 28, 2002 to Public Docket OPP-30000-60. (EPA MRID No. 45622311).

Epidemiological Evaluations

Triazine Urine Monitoring Program. Baranyai, (1994). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598605).

An Evaluation and Critique of Atrazine Developmental Toxicity Safety Evaluations and Human Epidemiological Data: A Review of Published and Unpublished Studies for Hazard Potential and Risk Estimation. Johnson, (1993). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598608).

A Critique of a Document Entitled "Summary: Low Birth Weight in Relation to Source and Characteristics of Drinking Water Supplies in Rural Areas of Iowa" by Peter Isacson, Dated October, 1989. Austin, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598609).

The Effects of Representative Groundwater Pesticides on Reproduction and In Utero Development of Experimental Animals. Johnson, (1993). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598610).

An Evaluation of Epidemiologic Studies of Exposure to Triazines and Cancer in Humans. Delzell and Sathiakumar, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598625).

Mortality Among Workers at Two Triazine Herbicide Manufacturing Plants. Sathiakumar and Delzell, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598626).

Response to EPA Review of Retrospective Triazine Mortality Studies, Breckenridge, (1995). Submitted March 23, 1995 to Public Docket OPP-30000-60 (EPA MRID No. 43598627).

An Updated Follow-Up Study of Workers at the CIBA-GEIGY McIntosh Plant Submitted April 16, 1997 to Public Docket OPP-30000-60. (EPA MRID No. 44256501)

A Combined Analysis of Mortality Among Workers at CIBA-GEIGY's McIntosh and St. Gabriel Plants - An Update. Submitted April 16, 1997 to Public Docket OPP-30000-60 (EPA MRID No. 44256502)

A Follow-Up Study of Workers at the Ciba-Geigy St. Gabriel Plant. Submitted April 25, 1996 to OPP-30000-60.

A Follow-Up Study of Mortality Among Workers at the Novartis St. Gabriel Plant. Submitted June 12, 2000 to Public Docket OPP-30000-60. (assigned 2 EPA MRID Nos. 45152101 and 45154901 by mistake)

Follow-Up Study of Cancer Incidence Among Workers in Triazine-Related Operations at the Syngenta St. Gabriel Plant. Study No. 2207-01. Submitted to Public Docket OPP-30000-60 October 12, 2001. (EPA MRID No. 45518401)

An Evaluation of the Report by Dr. Delzell el al, on A Follow-Up Study of Cancer Incidence Among Workers in Triazine-Related Operations at the Novartis St. Gabriel Plant. Study No. 1813-02 Submitted July 24, 2002 (EPA MRID No. 45727301)

A Nested Case-Control Study of Prostate Cancer and Atrazine Exposure. Study No. 3001-03. Submitted October 7, 2003. (EPA MRID No. 46089401).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

HED DOC. NO 014431

MEMORANDUM

DATE: December 13, 2000

SUBJECT: Atrazine: Evaluation of Carcinogenic Potential

FROM: Karl P. Baetcke, PhD
Vicki Dellarco, PhD
Health Effects Division (7509C), Office of Pesticide Programs

THROUGH: William Burnam, Chairman, Cancer Assessment Review Committee
Health Effects Division (7509C), Office of Pesticide Programs

TO: Sanjivani Diwan, PhD
Executive Secretary, Cancer Assessment Review Committee
Registration Branch 4
Health Effects Division (7509C), Office of Pesticide Programs

This memorandum contains the conclusions from the seventh Health Effects Division (HED) Cancer Assessment Review Committee (CARC) meeting (December 13, 2000) subsequent to the sixth CARC meeting held in November 2000 (Memorandum, From Roger Hawks to Catherine Eiden, November 1, 2000). The conclusions from the November 1, 2000 meeting were considered provisional pending receipt and review of the written comments from the June 27th to 29th, 2000 FIFRA Scientific Advisory Panel (SAP) meeting which convened to consider a preliminary hazard and dose-response assessment for atrazine prepared by HED[1]. The final report of the June 27, 2000 SAP meeting is now available[2]. Thus, the purpose of the December 13, 2000 CARC meeting was to consider and revise, if necessary, and finalize provisional conclusions of the November 1, 2000 CARC meeting.

[1]see http://www.epa.gov.scipoly/sap/2000/june27finalparta_atz.pdf
[2]see http://www.epa.gov.scipoly/sap/2000/june27/finalatrazine.pdf

CARC members present:

Lori Brunsman

Joycelyn Stewart

Clark Swentzel

Mike Ioannou

Vicki Dellarco

Karl Baetcke

Virginia Dobozy

Marion Copley

Bill Burnam

Linda Taylor

Others present:
Cathy Eiden
(HED - nonvoting)

EXECUTIVE SUMMARY

At a meeting of the CARC held on December 13, 2000, atrazine was classified as "Not Likely To Be Carcinogenic To Humans" in accordance with the draft Guidelines for Carcinogen Risk Assessment (July, 1999). This decision was based on the information discussed below.

Atrazine is associated with mammary and pituitary tumors in female Sprague-Dawley (SD) rats, but not in male SD rats, or either sex of Fischer 344 (F-344) rats or CD-1 mice. Mutagenic and estrogenic activity do not appear to play a significant role in atrazine-associated carcinogenicity. Biological plausibility has been established for the mode of carcinogenic activity of atrazine. The rat cancer mode of action (MOA) involves a process consisting of modulation of the gonadotrophin releasing hormone (GnRH) pulse, attenuation of pituitary releases of luteinizing hormone (LH), and alteration of ovulatory cycles, expressed as constant estrus, which leads to prolonged exposure of mammary and pituitary tissues to estrogen and prolactin, and development of tumors in response to the prolonged hormone exposures. This MOA essentially accelerates the normal aging process in female SD rats. It would be expected to be operative in other rat strains with a similar reproductive aging process (e.g. Long Evans and Wistar). Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen and prolactin) that is found in SD rats is not expected to occur in humans. Instead, humans respond to reduced LH by having reductions in estrogen and prolactin. Although possible associations between atrazine exposure and non-Hodgkins lymphoma (NHL) and ovarian cancer have been reported in a few epidemiology studies, there is no supporting evidence or a sound argument of biological plausibility that these cancers may result from exposure to atrazine. Also, the lack of multiple confirming studies indicates that the human investigations by themselves do not make a strong case for an association between atrazine exposure and human cancer.

I. INTRODUCTION

At the meeting of the CARC held on December 13, 2000, the final report of the Scientific Advisory Panel (SAP Report No. 2000-05) was considered along with the provisional conclusions reached in the previous meeting of the CARC (November 1, 2000). The major conclusions of the SAP were as follows:

1. High doses of atrazine cause an increased incidence and earlier appearance of mammary adenomas and carcinomas in female SD rats but not in female F-344 rats, male SD or F-344 rats, or CD-1 mice of either sex.

2. Atrazine's MOA for the development of mammary tumors has been demonstrated. The SAP pointed out the uncertainties in the MOA but concluded that the weaknesses and limitations have been adequately addressed and are not sufficient to raise doubt about the overall MOA.

3. Regarding the question of relevance of the MOA in rats to humans, the SAP concluded:

a) There are similarities in the control of the hypothalamic-pituitary-ovarian axis between

humans and rats but there are important differences. The MOA for mammary tumors in SD rats is an acceleration of the reproductive aging process in which decreased LH levels lead to prolonged exposure of mammary tissue to estrogen and prolactin. In contrast, reproductive aging (menopause) in human females is characterized by low levels of estrogen and high levels of LH and follicle stimulating hormone (FSH).

b) There was some concern about epidemiology studies demonstrating a possible increased risk of NHL and ovarian cancer associated with atrazine exposure. However, the Panel concluded there was not a strong association due to the lack of multiple studies and some inconsistencies in the reported studies.

c) The Panel concluded that hypothalamic amenorrhea (HA) and polycystic ovarian syndrome (PCOS), anovulatory conditions in human females proposed by EPA as possible correlates to the reproductive effects of atrazine in rats, present much different endocrine profiles than age-related persistent estrus in SD rats.

4. It was the consensus of the SAP that atrazine should be classified as either "Not Likely to be Carcinogenic To Humans" or "Not Enough Information to Classify". The Panel also concluded that the MOA for atrazine carcinogenicity is not applicable to developing fetuses and children.

A preliminary hazard and dose-response assessment that was presented to the SAP (June 27, 2000) concluded that atrazine should be classified as "Likely To Be Carcinogenic To Humans." The "Likely" cancer classification was proposed because there is some evidence in the literature that CNS-acting drugs, like atrazine, may disrupt the GnRH and LH pulses and lead to disruption of the menstrual cycle in primates and humans. Further, it was thought that conditions of anovulation in humans, although in several respects dissimilar to atrazine's mode of action in the SD female rat, raised uncertainties about the possible endocrine imbalance by this CNS mode of action. Therefore, it was proposed to the June 27th SAP that human relevance should be presumed. However, as noted above, the June 27th SAP expressed the view that the mode of carcinogenic action of atrazine is not expected to be operative in humans and that atrazine should not be classified as a "Likely" human carcinogen but that "it would be more appropriate to classify atrazine as either "Unlikely To Be a Human Carcinogen" or "Not Enough Information To Classify." At the November 1, 2000 CARC meeting, the view of the SAP was discussed and atrazine was reclassified, subject to review of the final SAP report, as "Not Likely To Be Carcinogenic To Humans." Below is a reconsideration of the November CARC conclusions in light of the SAP final report.

II. EVALUATION OF CARCINOGENICITY

In reaching a final decision on the carcinogenicity classification for atrazine, the committee considered the following information.

1. Data demonstrating an increased incidence and decreased time to onset of mammary and pituitary tumors in female SD rats, but not in male SD rats or F-344 rats or CD-1 mice of either sex.

2. Data on the proposed MOA associated with the carcinogenesis seen in female SD rats following atrazine exposure.

3. Comments provided in the final report of the SAP meeting of June 27, 2000 regarding the relevance of the MOA established for rat carcinogenicity to humans.

4. Evidence that mutagenicity and direct estrogenic activity do not play a significant role in atrazine-associated carcinogenicity.

5. Results of epidemiology studies that suggest an association between atrazine exposure and carcinogenicity in humans.

III. COMMITTEE'S ASSESSMENT OF THE WEIGHT OF EVIDENCE

The following factors were considered in evaluating the weight of evidence.

A MOA has been established for these mammary and pituitary tumors in female SD rats that is unlikely to be operative in humans. Previously the CARC classified atrazine as a "Likely" carcinogen and the draft document presented to SAP June 27, 2000 reflected this opinion. This classification assumed that a pair of human models of anovulatory conditions associated with aberrant GnRH pulses (PCOS and HA) were models of the above-described rat MOA in humans. The deliberations at the June SAP meeting clearly reflected the SAP's view that these two human models were not appropriate for comparison to the SD rat model and did not establish the human relevance for the proposed mode of action. GnRH pulse modulation of pituitary releases of LH is a central driver of ovulation in the SD female rat, and atrazine is essentially accelerating the aging process of the CNS control of ovulation, which leads to a constant state of estrus (anovulation), and prolonged exposure to estrogen and prolactin. As noted by the SAP, although there are certain similarities in the control of the hypothalamic-pituitary- ovarian axis between humans and rats in that the hypothalamus can play a key regulatory role in primates, there are fundamental differences. Unlike the SD rat, CNS modulation is not the driving factor on human GnRH and LH releases. The EPA preliminary atrazine hazard and dose-response assessment wrongly assumed that an increase in estrogen could result from an attenuation of the LH release in humans. Although human conditions of anovulation are associated with aberrant GnRH and LH pulsatile releases and even if atrazine induced anovulation in humans like in the SD rat, there is no evidence for the potential of an unopposed estrogen condition in humans that would lead to tumor development. It appears that in humans when LH is low, such as in HA, a state of low serum estrogen is found, not elevated or prolonged estrogen exposure. There is no known cancer risk associated with HA patients, albeit they are at risk to a number of other clinical conditions (e.g., osteoporosis, heart disease, infertility). Another condition of anovulation, PCOS, is also not a good model for atrazine cancer MOA in SD rats. The etiology of PCOS is multi factorial, and LH secretion is elevated due to increased synthesis of androgen and its conversion to estrogens. Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment

conducive to tumor development (i.e., elevated or prolonged exposure to estrogen or prolactin) that is found in SD rats is not present in humans. Therefore, it is unlikely that atrazine's mode of cancer action in SD rats is operative in humans. The CARC agreed with the view reflected in the written comments of the June 2000 SAP review.

The human epidemiology database does not provide sufficient evidence to associate atrazine with human cancer of any tissue. The SAP report contains a discussion of issues regarding the Agency's evaluation of the human epidemiology data on atrazine and recommendations for further analyses of the data. Despite some of the short-comings pointed out by the panel, the panel stated that the summary paragraph on the evaluation of the human epidemiology in the Agency's assessment document should be revised to:

> "To summarize, there are a few epidemiological studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies available indicates that the human studies by themselves do not make a strong case for an association."

On closer evaluation since the June SAP meeting, the CARC agreed with the SAP that the human studies "by themselves do not make a strong case" for an association between atrazine exposure and a cancer risk. Although possible associations between atrazine exposure and NHL and ovarian cancer are reported, there is no supporting evidence or a sound argument of biological plausibility that these cancers may result from exposure to atrazine. Several two- year bioassays with atrazine in SD and F-344 rats, and CD-1 mice failed to show evidence of an increased incidence of ovarian tumors or lymphomas. Furthermore, ovarian cancer is associated with frequent ovulations (not anovulation) or stimulation by FSH and LH (not suppression of LH), thus increasing their exposure to estrogens (see Fathalla, M.F., 1971, Lancet 2 (7716):163; Cramer, D.W. and Welch, W.R., 1983, J. Natl. Cancer Inst. 71(4):717-21). NHL is associated with immune dysfunction and not hormonal imbalance.

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL

Following discussion of the conclusions reached at the November 1, 2000 CARC meeting and consideration of the comments and recommendations provided by the Scientific Advisory Panel, the December 13, 2000 CARC reaffirmed the classification of atrazine as "Not Likely To Be Carcinogenic To Humans" based on the overall weight of evidence that:

1. The mode of carcinogenic activity in the female SD rat is supported by the data.

2. The mode of carcinogenic activity in the female SD rat essentially involves an acceleration of the reproductive aging process.

3. The mode of action for the carcinogenicity of atrazine is unlikely to be expressed in humans; no human conditions can be established that support a potential for atrazine to lead to carcinogenicity in humans.

4. Other modes of action are not supported by the available data and, in particular, mutagenic and estrogenic activity do not appear to significantly contribute to atrazine's carcinogenic potential.

5. Although a few epidemiological studies suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer, these cancers do not appear to be plausible based on atrazine's mode of action. Therefore, the human studies by themselves do not make a strong case for an association.

The CARC agreed that a response to the SAP comments, the classification of atrazine as "not likely to be a human carcinogen", and the supporting weight of evidence for the classification should be incorporated in the atrazine hazard and dose-response assessment document when it is finalized.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

October 28, 2003

MEMORANDUM

SUBJECT: Review of Atrazine Cancer Epidemiology
DP Barcode D295200, Chemical #080803

FROM: Jerome Blondell, Ph.D., Health Statistician
Chemistry and Exposure Branch
Health Effects Division (7509C)

Vicki Dellarco, Ph.D., Senior Science Advisor
Health Effects Division (7509C)

THRU: Francis B. Suhre, Chief
Chemistry and Exposure Branch
Health Effects Division (7509C)

Margaret Stasikowski, Director
Health Effects Division (7509C)

TO: Eric Olson, Ph.D., PM Team Reviewer
Michael Goodis, Product Manager
Special Review and Reregistration Division (7508C)

This review summarizes scientific studies related to atrazine and cancer epidemiology including a consideration of animal mode of action related to selected cancers. The review starts with an examination of animal mode of action issues related to prostate and ovarian cancers and non-Hodgkin's lymphoma. This section is followed by a review of EPA findings concerning prostate cancer in a manufacturing plant, the Agricultural Health Study, and two California studies. The following section addresses the evidence concerning non-Hodgkin's lymphoma and then, other cancers. The review concludes with a summary of three published reviews of atrazine and cancer epidemiology published 1996-1999, followed by a listing of those studies published since 1999 and considering their impact on the weight-of-evidence. In order to be comprehensive all known epidemiologic studies of atrazine and triazines have been included.

The U.S. Environmental Protection Agency (EPA) requested a review of atrazine and

prostate cancer by the Scientific Advisory Panel (SAP) made up of outside experts. A report of the
SAP findings (meeting minutes) from the July 17, 2003 meeting can be found at http://www.epa.gov/scipoly/sap/. This review considers the SAP findings and, where appropriate, revises the Agency's findings related to atrazine and prostate cancer. This review also explains why studies of other cancers where not presented to the Panel at the July 2003 meeting. To assist the reader, an outline of this review is provided below:

I. Examination of prostate and ovarian cancers and NHL in the context of the animal mode of action
II. Atrazine and prostate cancer epidemiology
    A. Prostate Cancer - Manufacturing plant study
    B. Prostate Cancer - Agricultural Health Study in Iowa and North Carolina
    C. Prostate Cancer - Pesticide use data and cancer incidence in California counties
    D. Prostate Cancer - Overall conclusion
III. Atrazine and epidemiology of Non-Hodgkin's Lymphoma
IV. Atrazine and epidemiology related to other cancers
V. Results of other reviews of cancer and atrazine through 1999
VI. Studies published since 1999 or not included in the three published reviews
VII. EPA Conclusion: Atrazine exposure and NHL and other cancers

I. Examination of prostate and ovarian cancers and NHL in the context of the animal mode of action

As discussed in the January 31, 2003 Interim Reregistration Eligibility Decision, atrazine's mode of action (i.e., a decrease LH surge, failed ovulation and estrous cycle disruption) for induction of mammary gland tumors (the only tumor observed in animal bioassays) in SD female rats is not considered relevant to humans. Because the epidemiology literature on atrazine (triazines) report that atrazine exposure may be associated with an increased incidence of prostate and ovarian cancers and NHL, the available chronic toxicity animal bioassays were closely examined for any indication of either prostate, ovarian cancer or NHL, as well as the etiology of these tumor types.

The prostate glands in male laboratory animals do not appear to be a target of atrazine toxicity. Subchronic and chronic rodent and dog studies and the multigeneration rat studies conducted for atrazine and its major metabolites have not demonstrated treatment related tumors or prostatitis and only shown inconsistent changes in prostates weights. The SD and Fisher rats and CD-1 mice are poor models for evaluating prostate cancer. Atrazine has been shown to result in prostatic inflammation of the adult rat offspring (Stoker et al., 1999) but this is not due to direct treatment of the offspring, but rather treatment of the dams. In this case, atrazine leads to a decrease in early lactational exposure to prolactin (via treatment of the mothers). Alterations in neonatal prolactin regulation lead to hyperprolactemia, which in turns lead to the prostatic inflammation found in the adult offspring. This work supports the neuroendocrine

mode of action for atrazine rather than a mode of action that would explain prostate cancer in adult male workers. Although the etiology of prostate cancer is not clearly understood, the hormonal changes caused by atrazine would be in the opposite direction (i.e., decreased prolactin) of what would be expected for the development of prostate cancer. Furthermore, it has not been clearly established that prostatitis increases the risk to prostate cancer. Therefore, the animal data do not support a mechanism for atrazine contributing to the onset or promotion of prostate cancer in humans.

Ovarian tumors in most laboratory animals are a rare occurrence (Damjanov, 1989). A dose-related increase in ovarian tumor incidence is not seen in any study using atrazine, simazine or propazine, and in all studies, the incidences of ovarian tumors are (as would be expected) very low. Although the causes of ovarian cancer are not definitively known, the key events in the mode of action established for atrazine, i.e., decreased serum LH levels and a decreased number of ovulations over a lifetime, are the opposite of the events hypothesized to be associated with ovarian carcinogenesis. Some hypotheses have been advanced for ovarian carcinogenesis with the predominant hypotheses being the "incessant ovulation" hypothesis and the "gonadotropin" hypothesis (e.g., Fathalla, 1971). This hypothesis suggests that damage to the ovarian epithelium, resulting from frequent ovulations, leads to increased risk of cancer as this leads to increased epithelial cell proliferation. It should be noted that epidemiology studies show that factors which decrease the number of lifetime ovulations - such as pregnancy, breast-feeding and oral contraceptive use - reduce ovarian cancer risk (Berchuck and Carney, 1997). Therefore, animal data do not support a biologically plausible mechanism for atrazine contributing to ovarian cancer in humans.

There was not an increase in any dose group for a lymphoma of any type, including non-Hodgkin's lymphomas (NHL) in atrazine treated SD or F344 rats. The simazine chronic bioassay using both sexes of SD rat also failed to see any lymphomas in any animal in any dose group (McCormick, 1988). Likewise, no animal in either of the two groups examined (control and high dose tested - 1000 ppm) in the propazine chronic bioassay, had a lymphoma of any type (Jessup, 1980). The alterations in reproductive hormones that atrazine exposure is associated with have not been linked to an increased risk of NHL. NHLs are a broad group of neoplasias which originate from lymphoid tissues such as B-cells, T-cells and histiocytes, though the vast majority are B-cell in origin. The etiology of NHLs are unclear. Generally speaking, increased risk of developing NHL appears to be associated with conditions or xenobiotic exposures that result in immune dysfunction (Scherr and Mueller, 1996). An association between NHL and reproductive hormones such as LH, FSH, estrogens and prolactin, does not appear to be present. A mechanistic role for atrazine contributing to NHL has not been identified in laboratory studies.

In summary, multiple animal bioassays do not reveal an increased incidence of tumors at any endocrine site other than mammary gland in female SD rats. Other endocrine tumors that have been raised in epidemiological studies can not be biologically tied to atrazine's mode of action (i.e., decrease prolactin, decrease LH and suppression of ovulation). Thus, at this time, based on the available animal cancer and mode of action data and epidemiological studies, there is no tumor endpoint on which to base a cancer risk assessment for atrazine. EPA has considered

other possible modes of action (e.g., stimulation of aromatase activity) and finds that there are inadequate data to support these hypotheses. EPA's Office of Research and Development National Health and Environmental Effects Laboratory is currently conducting a detailed investigation of the effects of atrazine and related chlorotriazines on aromatase activity and steroidogenesis in an effort to determine whether or not these compounds can change estrone, estradiol and testostosterone synthesis in response to atrazine treatment in rats. As additional data become available, EPA will review them.

Berchuck A, Carney M. Human ovarian cancer of the surface epithelium. Biochem Pharmacol. 1997 Sep 1;54(5):541_4.

Bosland MC. Male reproductive system. In Carcinogenesis. Eds., MP Waalkes, JM Ward. New Your: Raven Press, Ltd., 1994, pp. 339-402.

Damjanov I., Ovarian tumours in laboratory and domestic animals. Curr Top Pathol. 1989;78:1_10.

Fathalla MF. Incessant ovulation__a factor in ovarian neoplasia? Lancet. 1971 Jul 17;2(7716):163.

Jessup, D. 1980. Two year oral chronic toxicity study in rats. IRDC study no. 382-007. MRID 00041408; Acc. No. 219502401.

McCormick, C.C. and Arthur, A.T. Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats. 1988. Study Number: 2-0011-09. MRID number: 406144-05. Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Scherr PA and Mueller NE, Non-Hodgkin's lymphomas, in Eds D. Schottenfeld and JF Fraumeni Jr.. Cancer Epidemiology and Prevention New York, Oxford University Press, 1996, pgs. 920-945.

Stoker, T.E., Robbinette, C.L., Cooper, R.L. 2000.Maternal exposure to atrazine during lactation suppresses suckling_induced prolactin release and results in prostatitis in the adult offspring. Toxicol Sci. 1999 Nov;52(1):68_79.

II. Atrazine and prostate cancer epidemiology

The following review addresses epidemiology related to prostate cancer, non-Hodgkin's

lymphoma, other cancers and includes a brief summary of earlier reviews and the most recently submitted studies related to atrazine and triazines.

A. Prostate Cancer - Manufacturing plant study

An epidemiology study was conducted of workers at the Syngenta St. Gabriel plant where atrazine is manufactured. That study reported a statistically significant increase in the incidence of prostate cancer among plant workers. The Agency, upon review of this study, requested additional information on the exposure profile of the employees diagnosed with prostate cancer and this information was provided and reviewed. To further analyze the question of exposure a nested case-control study was proposed by Syngenta and conducted for them by Health Practice Exponent Inc. (Hessel et al. 2003). Preliminary results of this study, a review by the Agency, comments from four external peer reviewers, a Syngenta-sponsored expert panel review, and comments by the Natural Resources Defense Council were provided to the EPA's Scientific Advisory Panel in July of 2003. EPA's view of the study was that the increase in prostate cancer observed in the St. Gabriel workers was probably due to the increase in PSA screening for these workers.

The Panel was requested to comment on the Agency's conclusion regarding prostate cancer and particularly the results from this study. The specific Agency conclusion that EPA asked the SAP to comment on was: "Due to the lack of a detailed exposure analysis based on job history and the limited statistical power due to the small sample size, atrazine could not be ruled out as a potential cause but a role for atrazine seems unlikely."

The Panel's analysis of the St. Gabriel study differed to a degree from the Agency's conclusion. The SAP did conclude that "the increase in Prostate Specific Antigen (PSA) screening at the St. Gabriel plant likely led to an increase in the detection of cases of prostate cancer." Further, the Panel noted that "[s]ubstantive and persuasive arguments have been made to support the EPA's conclusion that PSA screening could explain the observed increase in prostate cancer incidence in the workers." Nonetheless, the Panel did not believe there was sufficient evidence to conclude that it was "unlikely" that atrazine had a role in the increased prostate cancer cases "given the severe limitations of the St. Gabriel study, particularly those pertaining to small sample size, questionable exposure assessment and lack of an appropriate comparison group." According to the SAP, PSA screening may be only a "partial explanation" for the increase in prostate cancer and that "atrazine cannot be ruled out as a potential cause."

The Agency agrees with the SAP's analysis and has rewritten its conclusion as follows:

> The increase in prostate cancer incidence at the St. Gabriel plant in Louisiana is consistent with the intensive PSA screening. This is because prostate cancer was found

> primarily in active employees who received intensive prostate specific antigen (PSA) screening, there was no increase in advanced tumors or mortality, and proximity to atrazine manufacturing did not appear to be correlated with risk. No evidence was identified that permit a determination that some of the increase was likely due to exposure to atrazine although atrazine exposure cannot be ruled out at this time. However, the study was insufficiently large and suffered from other limitations that prevent a determination that all of the increase in prostate cancer was probably due to the intensive screening program.

One of EPA's external reviewers agreed with this finding regarding the role of PSA screening. Dr. Edward Giovannucci of the Harvard School of Public Health stated, "Thus, the increased excess of prostate cancer observed in the Novartis study is compatible with increases expected in a population that is receiving intensive PSA screening." Another reviewer, Dr. Aaron Blair of the National Cancer Institute, though not in full agreement with Dr. Giovannucci, agreed that there was evidence to "suggest that PSA screening may well explain the excess incidence of prostate cancer in this cohort."

The Scientific Advisory Panel suggested that the Agency consider additional analysis of the St. Gabriel cohort. However, the resulting sample size would still limit the opportunity to draw further conclusions. The Agency questions whether additional analysis is warranted for other potential risk factors (such as smoking, diet and previous work history, and non-occupational or pre-employment exposure to triazine herbicides). Because of the way the study was designed this information is not available to investigators and it may not be feasible to obtain such information. The same applies to the suggested analysis of family history, history of prostate disease, (e.g., benign prostatic hypertrophy and prostatitis), and additional biologic samples that allow DNA extraction. The St. Gabriel study was a study based on available records and it might be difficult or impossible for investigators to obtain permission from all or most subjects or next-of-kin to get the additional information outlined by the SAP. The SAP repeatedly acknowledges "The St. Gabriel cohort study suffered from several limitations that could lead to negative findings in epidemiologic studies of similar design, particularly with regard to the very small sample size" which can greatly hinder the statistical power to detect an effect.

In October, 2003, Syngenta provided a completed report on the nested case-control study (cases and controls selected from within the cohort) by Health Practice Exponent Inc. that examined in more detail the exposure of 12 of 17 prostate cancer cases and examined the effect of screening on prostate cancer incidence (Hessel et al. 2003). EPA has not yet reviewed this study in depth, but a preliminary reading did not find any evidence that prostate cancers could be attributed to atrazine exposure. Statistical analysis suggest that PSA screening would explain some or all of the elevated rates of prostate cancer. The study authors concluded "There is no evidence for an association between atrazine exposure and prostate cancer among the workers at the Syngenta plant in St. Gabriel. The increased incidence of prostate cancer observed in the previous study could be explained by the PSA screening program."

B. Prostate Cancer - Agricultural Health Study in Iowa and North Carolina

Tied into the assessment of atrazine and prostate cancer is the recently published study Alavanja et al. (2003). This large prospective cohort study of 55,332 male pesticide applicators, known as the Agricultural Health Study, reported on the risk of prostate cancer and computed odds ratios for individual pesticides within the cohort. For atrazine the reported odds ratio (ratio of odds in favor of disease among exposed to the odds of disease among the unexposed, an odds ratio of 1.0 implies no increased risk from exposure) was 0.94 for ever/never use reported by questionnaire with a 95% confidence interval of 0.78 to 1.14. The Agricultural Health Study has a number of advantages over other epidemiologic studies of pesticides. It is the largest study of its kind, determines exposure prior to disease (thus, eliminating recall bias), analyzes a wide variety of potential and known confounders including other pesticide exposures, and has greater statistical power to detect small effects.

The Scientific Advisory Panel expressed concern that the use of ever/never use atrazine was "likely an inappropriate exposure metric" and that other factors such as measures of continuous or intermittent use should be considered. The Panel appeared to place little weight on the dose-response analysis based on cumulative exposure which combined duration, frequency, and intensity into one metric that did not show any association between atrazine and prostate cancer. The Agency agrees that other exposure metrics might be considered but disagrees that ever/never use and cumulative exposure is an inappropriate measure. Available pesticide usage data suggest that the pattern of use of atrazine as a preharvest herbicide limits variability in duration, frequency, and intensity of use and the dose-response analysis is a sufficient measure to account for this source of variation.

The Scientific Advisory Panel expressed concern that this study had a short follow-up period "with exposure information collected at the start of follow-up" and incorrectly stated this was less than five years. Because study subjects were queried about past as well as present use of atrazine, the follow-up period was much longer than five years. The National Cancer Institute is planning to redo the prostate cancer study with a much larger cohort next year when the sample size will be approximately twice as large. However, given the relatively tight confidence interval on the current estimate and the lack of any evidence of dose-response, the Agency does not expect the new study to produce results different from those already reported. Nevertheless, the Agency will revisit and revise these conclusions if the updated prostate cancer study produces different results suggesting a risk from exposure to atrazine.

C. Prostate Cancer - Pesticide use data and cancer incidence in California counties

Two studies were conducted in California which has maintained a population-based cancer registry since 1988 and a state-wide pesticide use reporting system. Mills (1998) obtained 1993 pesticide usage data for six pesticides with a suspicion of carcinogenicity based

on other toxicologic and epidemiologic studies. These data were compared using regression analysis with county age- and race- adjusted cancer incidence rates (1988-92). A borderline statistically significant correlation was found between atrazine usage and prostate cancer in black males, but not among Hispanic, White, or Asian males. This study is subject to aggregation bias because the exposure of individuals in the county was not measured. EPA considers such studies useful for guiding future studies, but not for reaching conclusions about causation.

A second study by Mills and Yang (2003) examined the effect of simazine rather than atrazine on prostate cancer among members of the United Farm Workers of America. The study found a borderline significant association between high simazine use and prostate cancer. Like the earlier Mills (1998) study, this study suffers from aggregation bias and a crude measure of exposure (total poundage of active ingredient by crop and county for a given time period) which may not reflect exposure among farmworkers, 90% of whom are not actively involved in applying or handling pesticides (1999-2002 data from presentation on National Agricultural Workers Survey by S. Gabbard, J. Nakamoto, and D. Carroll, September 24, 2003, funded by the Department of Labor). The use of total poundage of active ingredient by county was not normalized by number of workers and is especially problematic because it correlates with the size of the crop and acreage in the county and many other factors which might have little to do with exposure of farmworkers. For example, a county with double the poundage would be counted as having double exposure, even if it also had double the number of farmworkers and their exposure was the same in both counties.

D. Prostate Cancer - Overall conclusion

Studies of manufacturing and farming populations do not support a finding that atrazine is a likely cause of prostate cancer. The Scientific Advisory Panel stated that neither the Syngenta St. Gabriel Plant study or the Agricultural Health Study were "sufficient for EPA to conclude that there is no causal association between atrazine exposure and prostate cancer." However, the Agency does not find any results among these studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine.

III. Atrazine and epidemiology of non-Hodgkin's lymphoma

The National Cancer Institute has performed a number of studies of non-Hodgkin's lymphoma (NHL) in farming populations. The key findings related to atrazine are noted below.

A study by Schroeder et al. 2001 examined two subtypes of NHL for association with pesticide exposure based on earlier data from Iowa and Minnesota. The negative NHL subtype was significantly associated with 5 pesticides. For atrazine, the odds ratio was borderline significant (Odds ratio = 1.7 with 95% confidence interval 1.0-2.8). The Schroeder study cautiously stated "In conclusion, we found weak to relatively strong associations between many

agricultural exposures and t(14;18)-positive, but not t(14;18)-negative NHL." and "Causal relationships . . . are plausible, but associations should be confirmed in a larger study."

The study by Schroeder et al. (2001) contrasted with an earlier study by Zahm et al. (1993) conducted in these same two states plus Kansas and Nebraska which concluded, "In our judgment, these data provide little evidence that atrazine is associated with NHL among white men."

Added to these two conflicting studies is a study by DeRoos et al. (2003) published electronically in September 2003. EPA has not had time to review this study in depth, but did not find evidence sufficient to implicate atrazine as a likely cause of NHL. This study stated that "Reported use of several individual pesticides was associated with increased NHL incidence" but that "limitations of our data hinder the inferences we can make regarding specific pesticides". The hierarchical regression odds ratio (odds ratio adjusted for the effect of exposure to other pesticides) for atrazine was 1.5 (95% CI = 1.0 to 2.2) which, like the study by Schroeder et al (2001), has borderline significance. Studies with borderline significance increase the likelihood that chance or some confounder may be an explanation for the observed findings. The authors caution that "some of the positive results could be due to chance" though adjustment for the influence of other pesticides in the analysis makes this somewhat less likely.

Given the conflicting results and the extreme caution exhibited by the National Cancer Institute (NCI) in making its conclusion regarding specific pesticides, the EPA has concluded that evidence is not sufficient to implicate atrazine as a likely cause of non-Hodgkin's lymphoma. Nevertheless, EPA will consider these studies in conjunction with other evidence and may request additional external review. An exhaustive and thorough analysis of non-Hodgkin's lymphoma and pesticide use is planned by NCI for 2004-5. This analysis will include consideration of NHL subtypes and many other factors as well. Absent compelling information in the interim, EPA has determined that a thorough review of atrazine and NHL should be conducted when the NCI data are available.

IV. Atrazine and epidemiology related to other cancers

Other cancers besides prostate and non-Hodgkin's lymphoma were found to have an elevated, though not statistically significant, increase in risk at the St. Gabriel plant (Delzell et al. 2001). Other studies have suggested an increased risk for ovarian, breast, and other cancers. However, these studies are at best preliminary and should not serve as a basis for implicating atrazine as a human carcinogen due to their methodological limitations and the absence of replication in other populations. The National Cancer Institute is planning a review of atrazine and all types of cancers in 2004. Given the much larger sample size and strengths of this Agricultural Health Study, the Agency has decided further review of other cancers and atrazine should take place when results from this planned analysis are available. However, if other studies or additional compelling evidence becomes available in the interim, the Agency will expeditiously review the new evidence and the potential risk resulting from exposure to atrazine.

A summary of evidence from previous reviews of atrazine and cancer epidemiology, and studies published subsequently, are reviewed in the following section

V. Results of other reviews of cancer and atrazine through 1999

Three reviews of triazines including atrazine and cancer epidemiology have been reported in the 1996-1999 time period:

IARC. 1999. IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 73: Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. World Health Organization, Lyon, France.

Neuberger JS. 1996. Atrazine and/or triazine herbicides exposure and cancer: an epidemiologic review. *Journal of Agromedicine* 3(2):9-30.

Sathiakumar N, Delzell E. 1997. A review of epidemiologic studies of triazine herbicides and cancer. *Critical Reviews in Toxicology* 27:599-613.

These three reviews identified the 10 case-control studies (see numbered references at the end of this review, 1-10) and two published cohort studies of workers exposed to triazines at manufacturing plants (11-12).

Neuberger (1996) concluded "based on the data to date . . . there is no convincing evidence of a causal association between atrazine and/or triazine(s) and colon cancer, soft tissue sarcoma, Hodgkin's disease, multiple myeloma, or leukemia. . . .There is a suggestion of a possible association between atrazine and/or triazine(s) with ovarian cancer and non-Hodgkin's lymphoma. However, the ovarian cancer study needs to be replicated and the NHL studies fall short of providing conclusive evidence of risk because the results could be due to chance, bias, or confounding."

Sathiakumar and Delzell (1997) concluded "The available epidemiologic studies, singly and collectively, do not provide any consistent, convincing evidence of a causal relationship between exposure to triazine herbicides and cancer in humans."

The International Agency for Research on Cancer (1999) summarized the human carcinogenicity data as follows:

> A combined analysis of results of two cohort studies of agricultural chemical production workers in the United States showed decreased mortality from cancers at all sites combined among the subset of workers who had had definite or probable exposures to triazine. Site-specific analyses in this subset of workers yielded no significant

findings; a non-significant increase in the number of deaths from non-Hodgkin's lymphoma was seen, but was based on very few observed cases.

A pooled analysis of the results of three population-based case-control studies of men in Kansas, eastern Nebraska and Iowa-Minnesota, United States, in which the risk for non-Hodgkin's lymphoma in relation to exposure to atrazine and other herbicides on farms was evaluated, showed a significant association; however, the association was weaker when adjustment was made for reported use of phenoxyacetic acid herbicides or organophosphate insecticides. A sub-analysis of results for farmers in Nebraska, the State in which the most detailed information on atrazine use was available, showed no excess risk for non-Hodgkin's lymphoma among farmers who had used atrazine for at least 15 years, after adjustment for use of other pesticides. In a case-control study of non-Hodgkin's lymphoma among women in eastern Nebraska, a slight, nonsignificant increase in risk was seen. In all these studies, the farmers tended to have an increased risk for non-Hodgkin's lymphoma, but the excess could not be attributed to atrazine.

Less information was available to evaluate the assoication between exposure to atrazine and other cancers of the lymphatic and haematopoietic tissues. One study of Hodgkin disease in Kansas, one study of leukaemia in Iowa-Minnesota and one study of multiple myeloma from Iowa gave no indication of excess risk among persons handling triazine herbicides.

In a population-based study in Italy, definite exposure to triazines was associated with a two- to threefold increase of borderline significance in the risk for ovarian cancer. The study was small, and potential confounding by exposure to other herbicides was not controlled in the analysis.

Based on these findings the IARC concluded "There is inadequate evidence in humans for the carcinogenicity of atrazine." In a critique of IARC monographs, Huff (2002) questioned the decision on atrazine and animal carcinogenicity and based this partly on the Delzell et al. (2001) report showing increased risk for prostate cancer and above expected levels for certain cancers including buccal cavity (3 observed, 2.1 expected), esophagus (2 observed, 0.7 expected), stomach (2 observed, 0.9 expected), bladder (3 observed, 1.6 expected), thyroid (2 observed, 0.6 expected) and leukemia/lymphomas (7 observed, 4.5 expected). These data are based on Table 7 of the Syngenta report by Delzell et al. (2001). This table shows 9 different estimates of risk, not counting prostate (already discussed) and certain grouped categories. Therefore, 6 of 9 categories exhibited an excess, though statistically insignificant risk. Chance alone is a possible explanation for such findings. In addition, bias and confounding could produce such results. Therefore, these elevated, nonsignficant incidence ratios must be considered preliminary findings. Until these findings are replicated in other studies that address the serious methodological limitations (especially the low statistical power) of the present study, they should be regarded as spurious or suggestive at best. Therefore, the EPA disagrees with Huff (2002) that the epidemiology studies provide support for a revision of the IARC classification for atrazine.

Independent of these three reviews, EPA has performed internal reviews of all of the above studies which had statistically significant findings relevant to atrazine or triazines including additional updates to the manufacturing plant studies submitted to EPA but not published. With the exception of the possible association with ovarian cancer, which EPA reviewers stated needed to be confirmed in other populations, the Agency did not find convincing evidence of an association between triazines or atrazine and cancer.

Huff J. 2002. IARC monographs, industry influence, and upgrading, downgrading, and under_grading chemicals: a personal point of view. International Agency for Research on Cancer. *Int J Occup Environ Health*. 8(3):249-70.

VI. Studies published since 1999 or not included in the three published reviews

The following six studies were reviewed by EPA and submitted to the Scientific Advisory Panel for review in July 2003.

Alavanja MCR, Samanic C, Dosemeci M, et al. 2003. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol* 157:800-814.

Delzell E, et al. 2001. Cancer Incidence Among Workers in Triazine-related Operations at the Novartis St. Gabriel Plant" Oct. 12, 2001. MRID# 451521-01 and 455184-01, Chemical #080803. [Technical Report 170 pp.]

MacLennan PA, Delzell E, Sathiakumar N, et al. 2002. Cancer incidence among triazine herbicide manufacturing workers. *J Occup Environ Med.* 44:1048-1058.

MacLennan PA, Delzell E, Sathiakumar N, et al. 2003. Mortality among triazine herbicide manufacturing workers. *J Toxicol Environ Health A* 66(6):501-517.

Mills PK. 1998. Correlation analysis of pesticide use data and cancer incidence rates in California counties. *Arch Environ Health*. 53:410-3.

Mills PK, Yang R. Prostate cancer risk in California farm workers. 2003. *J Occup Environ Med*. 45:249-258.

Results from the Alavanja (2003), Delzell (2001), Mills (1998), and Mills and Yang (2003) have already been discussed above. The two reports by MacLennan et al. are updates to the two earlier reports by Sathiakumar (1992, 1995). Most of the results in these two studies are covered in much more detail by Delzell et al. (2001) which has already been discussed above in the sections on prostate cancer and other cancers. The mortality study (MacLennan et al. 2003) did find a borderline significant result for non-Hodgkin's lymphoma based on 4 observed deaths versus 1.1 deaths expected. The authors noted, however, that "one of the decedents whose death certificate included a diagnosis of NHL had medical records, including a biopsy report that indicated a diagnosis of poorly differentiated nasopharyngeal cancer. This case was not removed

from our analysis. To have done so would have introduced a bias because there is no satisfactory procedure for removing similarly misclassified cases from the numerator of general population mortality rates used to calculate the expected number of deaths. Our data were not of adequate statistical precision to demonstrate trends in NHL rates or SMRs by years worked and years since hire." This acknowledgment of bias based on a misclassified case means that borderline statistically significant finding would no longer be significant if the case were excluded. As stated above, this evidence is not sufficient to support a finding that atrazine is a likely cause of non-Hodgkin's lymphoma.

The following six studies, including two published since July 2003 meeting were not submitted to the Scientific Advisory Panel.

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF, Blair A. 2003. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med* 60:e11.(http://www.occenvmed.com/cgi/content/full/60/9/e11)

Hessel PA, Kalmes R, Smith TJ, Lau E, Mink P, Mandel J. 2003. A Nested Case-Control Study of Prostate Cancer and Atrazine Exposure. Final Report, October 3, 2003 performed by Health Practice Exponent, Inc. and submitted by Syngenta Crop Protection, Inc.

Hopenhayn-Rich C, Stump ML, Browning SR. 2002. Regional assessment of atrazine exposure and incidence of breast and ovarian cancers in Kentucky. *Arch Environ Contam Toxicol* 42:127-136.

Kettles MA, Browning SR, Prince TS, Horstman SW. 1997. Triazine herbicide exposure and breast cancer incidence: an ecologic study of Kentucky counties. *Environmental Health Perspectives* 105:1222-1227.

Schroeder JC, Olshan AF, Baric R, et al. 2001. Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma. *Epidemiology* 12:701-709.

Van Leeuwen JA, Waltner-Toews D, Abernathy T, et al. 1999. Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987-1991. *Int J Epidemiol.* 28:836-40.

DeRoos et al. (2003), Hessel et al. (2003), Schroeder et al. (2001) have already been discussed above. Hessel et al. (2003) is discussed at the end of the section on "Prostate Cancer - Manufacturing Plant Study". DeRoos et al. (2003) and Schroeder et al. (2001) are discussed in the section on non-Hodgkin's lymphoma.

The other three studies by Hopenhayn-Rich et al. (2002), Kettles et al. (1997), and Van Leeuwen et al. (1999) are ecological studies where the unit of analysis are populations or groups of people rather than individuals. An earlier study by Kettles et al (1997) suggested an association between triazine exposure and breast cancer in Kentucky. However, a later follow-

up study by Hopenhayn-Rich et al. (2002) did not support this finding for Kentucky and instead found results suggesting a protective effect for atrazine for ovarian cancer and no effect on breast cancer. The Hopenhayn-Rich et al. study was based on 5 year, age-adjusted cancer rates which are likely to be more stable than the two year rates used by Kettles et al. A study by Van Leeuwen et al. (1999) found a positive association between atrazine water contamination levels and stomach cancer among 40 ecodistricts in Ontario, Canada, and a negative association with colon cancer suggesting a protective effect for atrazine. The authors "noted that so-called 'ecologic studies', the type of analysis conducted in this research, have a number of weaknesses, including ecologic fallacy and multiple collinearity." Stomach cancer has been declining for many years and is likely associated with a number of dietary and lifestyle factors, not controlled for in the Van Leewen et al. (1999) study (Cancer Rates and Risks, 4th edition, National Cancer Institute 1996). All of these studies are subject to aggregation bias because the actual exposures of individuals in the county/district or how long they resided there is not known. As noted in standard epidemiology texts, ecologic studies "can suggest avenues of research that may be promising . . . In and of themselves, however, they do not demonstrate that a causal association exists" (Gordis L. Epidemiology. W.B. Saunders Company, Philadelphia, 1996). The authors themselves warn "conclusions concerning causality cannot be drawn" (Kettles et al. 1997). An ecologic study in Kentucky of similar design to those above, found a whole host of factors that vary across an urban-rural gradient (Blondell JM. Urban-rural factors affecting cancer mortality in Kentucky, 1950-1969. Cancer Detection and Prevention 11:209-223, 1988). Persons living in rural areas differ not only in terms of pesticide exposures, but also diet, parity, physical activity, exposure to viruses, and other lifestyle factors. Appropriate controls are critical when studying the relationship between pesticide exposure and cancer.

VII. EPA Conclusion: Atrazine exposure and NHL and other cancers

The Agency does not find any results among the available studies that would lead us to conclude that potential cancer risk is likely from exposure to atrazine. EPA plans to revisit this conclusion upon receipt of new studies, especially those from NCI's Agricultural Health Study on atrazine and all cancers, prostate cancer, and non-Hodgkin's lymphoma, all of which are planned for completion in the next 1-2 years.

Numbered references

1. Brown LM, Blair A, Gibson R, et al. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Research* 50:6585-6591.

2. Brown LM, Burmeister LF Everett GD, et al. 1993. Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control* 4:153-156.

3. Burmeister LF. 1990. Cancer in Iowa farmers: recent results. *Am J Ind Med* 18:295-301.

4. Cantor KP, Blair A, Everett G, et al. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Research* 52:2447-2455.

5. Donna A, Crosignani P, Robutti F, et al. 1989. Triazine herbicides and ovarian epithelial neoplasms. *Scan J Work Environ Health* 15:47-53.

6. Hoar SK, Blair A, Holmes FF, et al. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*. 256:1141-7.

7. Hoar SK, Blair A, Holmes FF, et al. 1985. Herbicides and colon cancer. *Lancet*. 1(8440):1277-8.

8. Hoar-Zahm S, Weisenburger DD, Babbitt PA, et al. 1988. Case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska (abstract). *Am J Epidemiol* 128:901.

9. Zahm SH, Wisenburger DD, Cantor KP, et al. 1993a. Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma. *Scan J Work Environ Health* 19:108-114.

10. Zahm SH, Weisenburger DD, Saal RC, et al. 1993b. The role of agricultural pesticide use in the development of non- Hodgkin's lymphoma in women. *Arch Environ Health*. 48(5):353-358.

11. Sathiakumar N, Delzell E, Austin H, Cole P. 1992. A follow-up study of agricultural chemical production workers. *American Journal of Industrial Medicine* 21:320-330.

12. Sathiakumar N, Delzell E, Cole P. 1995. Mortality among workers at two triazine manufacturing plants. *American Journal of Industrial Medicine* 29:143-151.

cc: atrazine file (080803)
Catherine Eiden (7509C)

Exposure to Atrazine through Drinking Water

Author: Peter Hertl, Ph.D.

Date: July, 19, 2004

Exposure to Atrazine through DW from Groundwater

GW Monitoring Programs

1. Atrazine and Total Chrlorotriazine (TCT) Data Sets

Atrazine and Total Chlorotriazine (TCT) levels in groundwater were determined in 2 Syngenta Studies (MRID No. 45399906, submitted 5/2/2001, MRID No.43934414, submitted 1/31/1996 and amendments MRID No. 43934414 submitted 2/20/1997, MRID No. 44049201, submitted 2/20/1997); the results are summarized in tables 1:

Table 1a: Community water Systems on Groundwater: Summary statistics representing 14863 CWS on GW (418 CWS with one or more atrazine detects in 93-98, 14445 with no atrazine detects in 93-98) in atrazine use areas assessed in 2000.

Percentile	Atrazine [ppb] Percentile of CWS	Atrazine [ppb] Percentile of Population of Persons served	TCT [ppb] Percentile of CWS	TCT [ppb] Percentile of Population of Persons served
95th	0.0235	0.0627	0.1134	0.2713
97.5th	0.0673	0.1726	0.3420	0.4940
99th	0.1719	0.3668	0.5624	0.6244

Table 1b: Rural Ground Waters Well Study: Study targeted 1505 vulnerable wells, primary sampling campaign in 1992-94, Summary Statistics

Percentile	Atrazine [ppb]	TCT [ppb]	Well Selection Criteria included:
95th	0.34	1.99	• high atrazine use areas for the State; • hydrologically vulnerable areas with shallow water tables and permeable soils; • previous detections of atrazine; • proximity to a field where atrazine had been used • permission to sample was granted by the owner.
97.5th	0.75	3.39	
99th	2.10	8.70	

The studies demonstrate that:

- In CWS on GW atrazine and TCT levels will not exceed EPA's atrazine MCL of 3 ppb or EPA's DWLOC of 12.5 ppb for TCT in finished water;
- Based on EPA's review of these study the agency concluded that *"exposure to atrazine and its metabolites from GW CWS is low and limited"*
- In the primary 92-94 sampling campaign on vulnerable rural wells: eight out of 1,505 wells (0.5%) had atrazine concentrations above the MCL of 3 ppb, seven wells (0.5%) had total chlorotriazine concentrations exceeding 12.5 ppb in the 1992-1994-time period. 1,145 out of 1,505 wells (76.1%) had less than 0.1 ppb atrazine in the well water.

The 92-94 and other historical study results must be put in context to actual changes in labels and use practices. The groundwater related label changes made in 1990 prior to the first sampling campaign in 1992-94 were:

- Reduction in maximum rate allowed for non-cropland total vegetation control from 40 lbs. ai/a to 10 lbs. ai/a;
- Rate reductions in corn/sorghum (4 to 3 lbs ai/a);
- Restricted use classification for ground water concerns added (excluded uses for lawn care); 50 ft. Well-head buffer added for mix/load/use.

In 1992 additional label changes were added with the aim to further reduce groundwater detection levels. They included:

- Deleted non-cropland total vegetation control (@ 10 lbs. ai/a rate)
- Reduction in corn/sorghum rates from a maximum of 3 lbs. ai/a/year to 2.5 lbs ai/a/year (combined pre- and post-use) or maximum of 2.0 as single pre-emergence or post-emergence treatment;
- State/local preemption for more stringent requirements to allow use of localized best management practices (BMPs)

Since it a) usually takes a few years for groundwater related mitigations to take effect (it typically takes 2-3 years for product inventories to clear) and b) residue transport to groundwater tends to take a minimum of 2 years, the 1992-4 sampling campaign most likely did not show the full impact of Groundwater residue reduction resulting from these restrictions. More recent sampling campaigns of Syngenta's Rural Well Study program, however, demonstrate the effect clearly.

A first re-sampling campaign was performed in 1994-1997 to gauge the effects of atrazine label changes and Best Management Practices. The time interval of a minimum of 4-7 years would be expected to allow an estimate to be made of the impact the 1990 label changes, but was probably less likely to demonstrate the additional reduction brought forward by the 1992 changes.
For this campaign, a total of 104 wells in several States were re-sampled. These wells were selected by the individual States from the population of wells in the original sample. The sub-sample was based on the States expert judgment and knowledge of vulnerable areas.

The high percentile statistics for the 1st and 2nd sampling campaigns are summarized in Table 2a, individual data points are presented in Figure 2b. The average time interval between repeat samples was 20 months (range: 5 - 48 months). The data showed a general decline in concentrations of atrazine and total chlorotriazines, especially in the most vulnerable areas, where groundwater levels are quicker to respond.

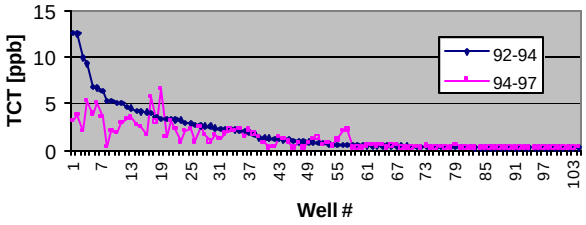
Table 2a: Upper percentile Concentrations in 104 rural wells sampled during (92-94) and (94-97)

Upper percentile Concentrations in 104 rural wells sampled during (92-4) and (94-97)

Percentile	Atrazine 92-94 [ppb]	TCT 92-94 [ppb]	Atrazine 94-97 [ppb]	TCT 94-97 [ppb]
90	2.20	6.68	0.76	3.13
95	4.20	12.51	0.96	3.73
99	8.72	14.72	1.49	5.73

Decline in high exposure wells significant for **parent and TCT**

Table 2b: TCT Residues in Rural Wells 92-94 and 94-97



In 2001 Syngenta initiated a second re-sampling campaign for the fourteen wells that had previous detections of either atrazine above MCL and/or total chlorotriazine exceeding HED's proposed Drinking Water Level of Concern (DWLOC, 12.5 ppb).

Analytical results from the 2001 survey indicated that the levels of atrazine and its chlorinated metabolites have declined significantly in all of the wells since the original survey. Most importantly, none of the 14 wells exceeded either the atrazine MCL (3.0 ppb) or the total chloro-triazine DWLOC for the most sensitive sub-population group (i.e., infants at 12.5 ppb). Exceedence frequency of non-compliant wells decreased from 0.7% and 0.5% in 92-97 to 0% and 0% in 2001. Results for the 92-97 campaigns and the 2001 re-sampling are summarized in Table 3.

Table 3: Study	No. Wells Represented	No. of Wells Analyzed	No. (%) wells >0.1 ppb Atrazine	No. (%) wells >1 ppb Atrazine	No. (%) wells >3 ppb Atrazine	No. (%) wells>6 ppb Atrazine	No. (%) wells >12.5 ppb TCT
Rural Well Study (RRW) 92-97	1505	1505	360 (23.9%)	48 (3.2%)	8 (0.5%)	3 (0.2%)	7 (0.5%)
Resampling 2001 wells >3 Atra zine or > 12.5 ppb TCT in 92-94	14	14	8 (57%)	4 (29%)	0 (0%)	0 (0%)	0 (0%)

The results indicate a decreasing trend of atrazine and TCT concentrations over time to levels below the MCL for atrazine or DWLOC for TCT even in the most vulnerable wells as a result of label mitigation and BMPs.

2. Atrazine Data Sets

The decreasing trend in atrazine concentrations observed in Syngenta's Groundwater studies was also confirmed by the monitoring results from:

1. Iowa Groundwater Monitoring Program (D. Kolpin et. al., "Temporal Trends of Selected Agricultural Chemicals in Iowa's Groundwater, 1982-1995: Are Things Getting Better?", J. Environ. Qual. 26:1007-1017, 1997). This study indicates a significant decreasing trend over the sampling period in both the frequency of atrazine detection and in median atrazine concentrations detected in Iowa's groundwater.

2. SDWA mandated Groundwater compliance monitoring programs conducted by the states since 1993.

Syngenta's Synoptic CWS Study (MRID No. 45399906, submitted 5/2/2001) confirms that the 99th Percentile atrazine level in the 418 CWS with previous SDWA detects in 93-98 declined from 1.53 ppb in 93-98 to 0.85 ppb in 2000. (see Table 1a above)

If the temporal trend of SDWA GW results is analyzed in terms of mean annual Atrazine concentrations and the number of CWS exceeding the MCL of 3 over time and over multi year periods the data are presented in table 4.

Table 4: Atrazine annual average and multi years period means in CWS on GW

Year	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Period Mean
Number of CWS w Data	3687	5851	7966	6538	7491	7225	5825	7496	5023	4858	7114	24505
Mean Annual Conc./ all CWS [ppb]	0.15	0.18	0.14	0.20	0.16	0.25	0.15	0.15	0.17	0.14	0.12	0.18
Max. Conc. P.a. [ppb]	4.20	5.26	5.56	10.40	2.88	3.63	2.60	3.80	1.53	1.87	2.10	2.72
Number of CWS Exceeding 3 ppb	1	7	4	8	0	2	0	2	0	0	0	0

Annual mean concentrations in finished Groundwater are low and comparable to the concentrations measured in Syngenta's Synoptic CWS survey (the fact thatslightly lower means were measured in the latter study is due to the lower LOQ's used in Syngenta's survey).
Annual maxima declined from a high of 10.4 ppb in 1996 to well below 3 ppb in 2001-3. In fact, none of the CWS on GW exceeded the MCL since 2000. There are no CWS on GW, which exceeded the 3 ppb chronically over the entire observation period.

3. Programs monitoring for Atrazine in GW in the 80's and early 90's

Syngenta's PLEX VI update (MRID No. 45253401, submission date 10/2000) provided an overview of Well Monitoring programs; the results are summarized in Table 5. All of the programs were conducted before the 1990/2 label changes were effectively reducing Atrazine concentrations in Groundwater and included drinking water as well as non-drinking water wells (as e.g. shallow agricultural monitoring wells and others).

Table 5: Summary of Non Syngenta Atrazine Monitoring Programs in Rural Wells.

Study	No. Wells Represented	No. of Wells Analyzed	No. (%) wells >0.1 ppb Atrazine	No. (%) wells >1 ppb Atrazine	No. (%) wells >3 ppb Atrazine
NAWQA	945	945	111 (11.7%)	23 (2.4%)	0 (0%)
USGS	150	150	8 (5.3 %)	0 (0%)	0 (0%)
Farm Bureau / Heidel-berg College Rural Well Project	12'151	12'151	601 (4.9 %)	108 (0.9%)	13 (0.1%)
Other Monitoring Programs	1'631	1'631			5 (0.3%)
Plex IV subtotal		**14'877**	**720 (5.4%)**	**131 (1.0%)**	**18 (0.1%)**
Alachlor Well Survey	6'040'000	1'430	33	3	

In these programs:

- About 0.1 % of the wells (incl. Non-drinking water wells) exceeded the MCL of 3 ppb in the late 80's and early 90's.
- The comparatively higher exceedence rate in Syngenta's 92-94 Rural Well programs (0.7% vs. 0.1%) validates its biased focus on worst-case use/exposure situations.

4. Monitoring for Atrazine in GW after the 1994 label changes and BMP's became effective:

In 1995-2001 the Acetochlor Registration partnership conducted a monthly sampling program in ~175 agricultural monitoring wells, not drinking water wells. The wells were placed very close to the edge of treated fields with a long history of corn use in highly vulnerable soil and groundwater settings and sampled the "first water" to ensure maximum sensitivity. They therefore **serve as worst case indicator wells** for potential concentration levels expected in rural drinking water wells. Yearly mean concentrations are summarized in Table 6:

Table 6: Summary of ARP Atrazine Samples in GW monitoring Wells

Annual Means (1995-99)	Atrazine <=0.1ppb	Atrazine >0.1 ppb	Atrazine >1.0 ppb	Atrazine > 3.0 ppb	Atrazine >6.0 ppb
No. of well years	651	192	14	2 1 well: 3.9/4.5 ppb	0
% of well years	77.22%	22.78%	1.66%	0.24%	0.00%
Total # of well years	843				

In the ARP GW monitoring program:

- Out of 175 wells one well exceeded 3 ppb in 2 years (0.2% of the well years)
- It is extremely unlikely that chronic Atrazine or TCT levels in high percentile wells would have exceeded the DWLOC of 12.5 ppb since TCT levels are typically 2 times the corresponding Atrazine high percentile concentrations.

Summary:

- **In GW CWS chronic long term Atrazine levels have never exceeded EPA's Atrazine MCL of 3 ppb and 99 percentile concentrations have been demonstrated to be significantly below EPA's MCL of 3 ppb for Atrazine and DWLOC of 12.5 ppb for TCT;**
- **Exceedence of the Atrazine MCL of 3 ppb and TCT DWLOC of 12.5 ppb in Syngenta's Rural Well program declined from 0.7 % for Atrazine and 0.5% for TCT in 92-94 to 0% in 2001, indicating exceedence of chronic Atrazine and TCT levels would be extremely unlikely even in the areas of greatest Atrazine use and highest well sensitivity.**
- **The frequency of exceedence in Syngenta's Rural Well sampling program was greater than in any other GW monitoring program conducted due to its deliberate selection of highly vulnerable settings – it is therefore a conservative worst case for estimating potential exposure through rural wells.**
- **Exceedence of the Atrazine MCL of 3 ppb in less focused Atrazine monitoring programs before the '94 label changes were effective was 0.1% or less, DWLOC exceedence is estimated at less than 0.1% - significantly lower than in Syngenta's worst case study – and should have further declined following the 1990/1992 label changes.**
- **Post '95 exceedence of the Atrazine MCL of 3 ppb in individual wells is 0.2% in ARP's worst-case edge-of-the field indicator – not drinking water - wells; exceedence of chronic TCT levels is extremely unlikely, since high percentile TCT levels are typically 2 times the corresponding Atrazine concentration and none of the well years exceeded 6 ppb in Atrazine .**

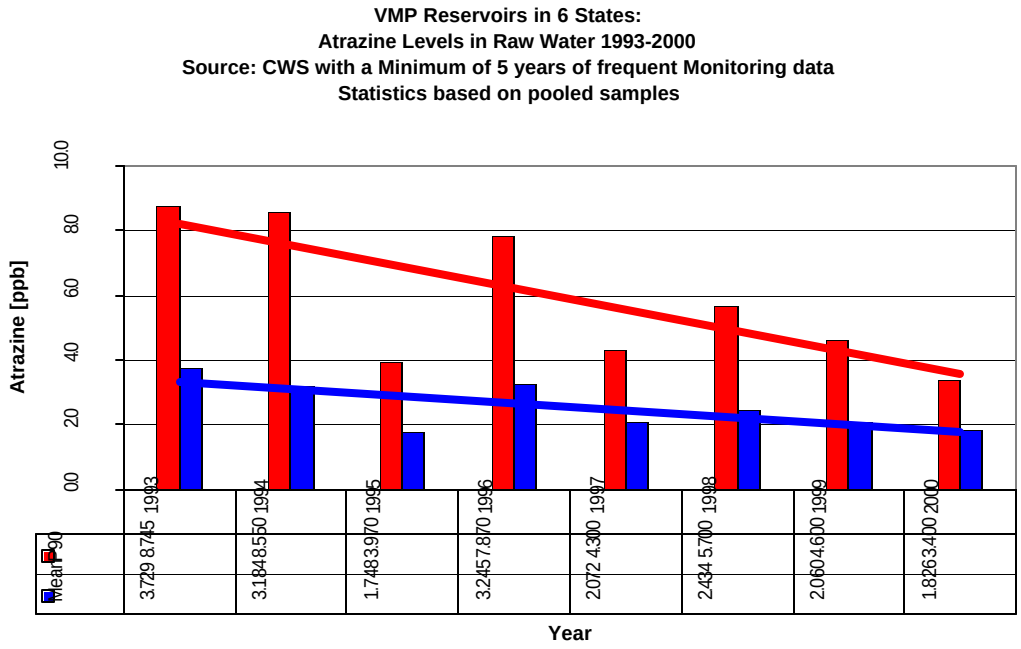
Therefore, it is confirmed that TCT levels resulting from non-point source exposure in rural wells will have a very low probability of ever exceeding the DWLOC of 12.5 ppb.

Exposure to Atrazine through DW from Surface Water

Numerous reports have been submitted to EPA's Atrazine docket containing datasets and statistical analyses describing the magnitude and temporal trend of Atrazine in surface water bodies used for drinking water production. The data sets included raw and finished water data on Atrazine generated by Syngenta under the "Voluntary Atrazine Monitoring Program", and data generated by Syngenta to investigate the co-occurrence of Atrazine residues and metabolite levels. Further data sets were the data generated by the states as mandated by the compliance-monitoring program mandated by the SDWA in finished water. Data analysis reports included regression functions that allow to predict TCT levels in raw water from the levels of measured Atrazine concentrations, a statistical rationale for a trigger concentration applied to the SDWA derived annual average concentrations in finished water that would allow to select systems which have a 1% or smaller probability to exceed 12.5 ppb in any 90 days interval within a year. Finally a set of analyses were reported investigating temporal concentration trends in CWS and flowing water bodies with the intent to understand if label changes in the early nineties, which were believed to be fully in effect in the second half of the nineties – had a significant effect on reducing exposure and frequency of MCL exceedence.
The SDWA data set can be described as a census of CWS on SW with any potential exposure to Atrazine whereas the VMP data set is a more frequent monitoring program focusing on the most vulnerable CWS in the high intense use areas.
Summary statistics of the data presented in these reports are given in figure 1 and table 7.

1. Syngenta's Voluntary Monitoring Program – Highly Exposed Systems
High frequency monitoring initiated 1993 with 20 CWS, expanded to 88 CWS in 1995 to 1997, 94 CWS in 1998, 100 CWS in 1999, and 01 CWS in 2000. A total number of 127 CWS in nine states was monitored in an 8-year period. There were 64 CWS in 6 States with > 5 years and > 6 months of data in each year. These 64 multi year data sets were analyzed by simple linear regression, using data points, percentiles and finally site-specific trend analysis investigating the trends on monthly concentrations.

Figure 1:



The data sets are temporally variable with higher levels usually occurring during the major use season followed by a decline throughout the season. Mean and high percentile raw water concentrations have been declining significantly and consistently in the 94-00 time period. Higher levels are typically triggered by a unique combination of watershed vulnerability, product use, and adverse weather patterns. Declining concentrations over time resulted in a decreased average raw water conc. of about 50% (3.7 to 1.8 ppb)

indicating a reduction of environmental exposure in these vulnerable surface water systems during the 94-00 time period.

2. Monitoring data sets generated under SDWA
SDWA mandated Surface water compliance monitoring programs were conducted by the states since 1993.
If the temporal trend of atrazine concentrations observed in vulnerable systems holds true for the universe of CWS in the Atrazine use areas it should also become apparent in finished water analyzed under data SDWA. Table 7 provides summary statistics for the 1993-2002 observation period.

Table 7: Atrazine annual average and multi year period means in CWS on SW

Year	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Period Mean
Number of SW CWS with SDWA Data	387	629	628	835	674	799	1001	856	1579	1392	1914	2148
Mean Annual Conc./ all CWS	0.37	0.65	0.48	0.61	0.56	0.47	0.38	0.34	0.25	0.27	0.20	0.29
Number of CWS Exceeding 3 ppb	5	28	3	23	15	3	2	1	2	1	3	3

Annual mean concentrations in finished Surface water for the universe of CWS monitored under SDWA are significantly lower than for the set of highly vulnerable systems monitored under VMP. Annual mean concentrations over all CWS have been well below the MCL since 1993. A limited number of CWS have exceeded 3 ppb in the in the mid-nineties, with 28 and 23 exceedences in 1994 and 1996, respectively. Since then both maximum annual average concentrations and the number of exceedences have declined significantly with a few sporadic exceedences per year. Over the entire monitoring period three systems exceeded the lifetime MCL of 3 ppb average over a multi year period.
SHIPMAN (IL1170950) – two exceedences in 94 and 96, Shipman has discontinued to operate as a DW facility due to overall quality issues with the raw drinking water source.
HWEA-CROFTON WATER DEPT (KY0240090) – one exceedence in 96, the CWS is now using groundwater and has not had an exceedence since.
HETTICK (IL1170500) – three exceedences 95, 96, 98 – the CWS has discontinued its operations due to overall quality issues with the raw drinking water source and is now purchasing water.

Therefore there is no significant exposure to atrazine to populations served from these three CWS.

Overall the SDWA data base supports the trends observed in the more focused VMP program: Raw and finished water Atrazine concentrations have declined significantly between the mid nineties and 2002/2003. The number of exceedences of 3 ppb in finished water from SW CWS is sporadic and typically 1-3 CWS per year. There are **NO** active CWS with period means exceeding the lifetime MCL chronically.

The conditions stipulated in the MOA identify CWS with a remote possibility to exceed EPA DWLOC by applying a conservative trigger to CWS annual atrazine average levels. Any CWS with an annual average concentration exceeding 2.6 ppb TCT (equivalent to approximately 1.6 ppb atrazine) will be included in a more frequent monitoring program to determine if exceedence actually occurs. Since 2003 we have 129 CWS in the AMP, today none of the sites has exceeded EPA's DWLOC in raw water or has received a notice of violation for exceeding the MCL in finished water. Any CWS that does exceed the DWLOC or the MCL in any one year will be put into a CWS site-specific management plan to ensure no future exceedences.

Summary:

- In the most vulnerable CWS on SW concentrations declined over time and resulted in a 50% decrease of annual mean concentrations (3.7 to 1.8 ppb) indicating a reduction of environmental exposure during the 94-00 time period.
- In the universe of CWS on SW mean concentrations in finished water decreased by more than 50% in the 1993-2003 period

- These data are supported by USGS data showing Atrazine levels in 53 Midwestern streams in 9 major use states decreasing about 50% between 89/90 and 94/95 – levels have continued to decrease in 98[1].
- There are **NO** active CWS with period means exceeding the lifetime MCL chronically.
- As part of the January 2003 atrazine re-registration requirements CWS with a potential to approach standards are required to be monitored and mitigated if levels exceed certain triggers – no system has exceeded EPA's DWLOCs since the program started.

[1] Scribner, E.A., Battaglin, W.A., Goolsby, D.A., and Thurman, E.M., 1999, Changes in herbicide concentrations in Midwestern streams in relation to changes in use, 1989-98, in Morganwalp, D.W., and Buxton, H.T., eds., U.S. Geological Survey Toxic Substances Hydrology Program--Proceedings of the Technical Meeting, Charleston, South Carolina, March 8-12, 1999–Volume 2 of 3--Contamination of Hydrologic Systems and Related Ecosystems: U.S. Geological Survey Water-Resources Investigations Report 99-4018B, p. 313-322